

# Agilent 2100 Bioanalyzer System

# **Maintenance and Troubleshooting Guide**



#### **Notices**

#### **Document Information**

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### In this Book

This manual provides maintenance and troubleshooting information for the Agilent 2100 Bioanalyzer system. It includes essential measurement practices, troubleshooting hints for hardware, software and applications, maintenance procedures and a list of spare parts and accessories.

This manual is based on the 2100 Expert Software revision B.02.08 and newer.

### Contents

1	Essential Measurement Practices 6
	Overview 7 Tools and Handling 8 Chip Priming Station 9 Reagents and Reagent Mixes 10 Samples 11 Chips 12 Agilent 2100 Bioanalyzer System 13
2	Troubleshooting the Instrument Communication 14
	Verify the Instrument Communication 15 Troubleshooting Communication Issues 17 Changing COM Port Settings 19 USB to Serial Adapter 20 How to create a Support Package 21
3	Troubleshooting the 2100 Expert Software 23
	Run Installation Qualification Test 24
4	Hardware Diagnostics 26
	Overview 27 Diagnostic Test Procedure 29
5	Troubleshooting the DNA Application 31
	Overview 32 Symptoms (DNA) 33 Symptoms (High Sensitivity DNA) 58
5	Troubleshooting the RNA Application 62
	Overview 63 Symptoms (RNA) 64

#### 7 Troubleshooting the Protein Application 83

Overview 84 Symptoms (Protein) 85 Symptoms (High Sensitivity Protein) 107

#### 8 Maintenance of the Electrode Cartridge 110

Overview 111

DNA and Protein Assays 112

RNA Nano Assay 114

RNA Pico or Small RNA Assay 118

How to Clean the Pin Set of the Electrode Cartridge 121

#### 9 Maintenance of the Chip Priming Station 126

Overview 127
Replacing the Syringe 128
Cleaning the Syringe Adapter 129
Replacing the Syringe Adapter 131
Replacing the Gasket 132
Checking the Chip Priming Station for Proper Performance - Seal Test 134

#### 10 Maintenance of the Agilent 2100 Bioanalyzer instrument 136

Overview 137 Cleaning the Lens 138 Changing the Fuses 139

#### 11 Maintenance of the Vortexer 144

Changing the Adapter 145

#### 12 Spare Parts and Accessories 146

Overview 147

### 1 Essential Measurement Practices

Overview 7
Tools and Handling 8
Chip Priming Station 9
Reagents and Reagent Mixes 10
Gel and Gel-Dye Mix 10
Samples 11
Chips 12
Agilent 2100 Bioanalyzer System 13

#### 1 Essential Measurement Practices

Overview

### Overview

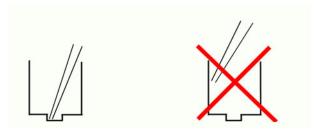
This section lists all user relevant hints on handling tools, chips, reagents and the Agilent 2100 Bioanalyzer system. For the latest information on assay-related hints, contact Agilent Technologies at:

https://www.agilent.com/en/contact-us/page

**Tools and Handling** 

### Tools and Handling

- Always follow the GLP-rules established in the laboratory.
- Always wear gloves when handling chips to prevent contamination.
- When pipetting sample, use non-filter pipette tips that are of adequate size. Pipette tips that are too large will lead to poor quantitation accuracy.
- Change pipette tips between steps to avoid cross-contamination.
- Always insert the pipette tip to the bottom of the well when dispensing liquid.
   Placing the tip at the edge of the well leads to bubbles and poor results.
   Holding the pipette at a slight angle will ensure proper dispensing of the liquid.



• Use a new syringe and electrode cleaner with each new kit.

#### 1 Essential Measurement Practices

**Chip Priming Station** 

### Chip Priming Station

- Refer to the appropriate Kit Guide for the correct position of the syringe clip and base plate.
- Replace the syringe with each new kit.
- Check the performance of the chip priming station by applying the seal test on a monthly basis. For details see "Maintenance of the Chip Priming Station" on page 126. If necessary, replace the gasket and/or adapter.

Reagents and Reagent Mixes

### Reagents and Reagent Mixes

- Handle and store all reagents according to the instructions given in the specific Kit Guide.
- Keep all reagents and reagent mixes (for example, the gel-dye mix) refrigerated at 4°C when not in use for more than 1 hour. Reagents left at room temperature may decompose, leading to poor measurement results.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes. Mix and spin down prior to use.

#### Gel and Gel-Dye Mix

- Use gel-dye mix within the specified time frame stated in the instructions from the kit guide. Otherwise, it may decompose and lead to poor measurement results.
- Protect dye and gel-dye mixes from light. Dye decomposes when exposed to light.

#### 1 Essential Measurement Practices

Samples

### Samples

- Refer to the assay specific Kit Guides for maximum allowed sample and salt concentration.
- For RNA assays: Heat denature all RNA samples and RNA ladder for 2 minutes at 70°C before use.
- For protein assays: Use 0.5 mL tubes for denaturation. Using larger tubes will lead to poor results.

#### **Essential Measurement Practices**

Chips

1

### Chips

- Prepared chips must be used within 5 minutes. Reagents may evaporate, leading to poor results.
- For DNA and RNA assays, vortex chips for 1 minute. Inappropriate and insufficient vortexing will lead to poor results. Use only the IKA vortexer for chip vortexing. Replace the chip adapter if it is worn out. For the MS-2 vortexers with 3 mounting screws, the replacement part number is 5065-9966. For MS-3 vortexers with 4 mounting screws, replacement adapters may be purchased directly from IKA (www.ika.de) with part number 3428300.
- Do not touch the wells of the chip. The chip could get contaminated resulting in poor measurement results.
- Do not leave any wells of the chip empty. The assay will not run properly. For DNA and RNA assays: Add 1  $\mu$ L of sample buffer to each unused sample well so the total liquid volume in each well is at least 6  $\mu$ L. For protein assays: pipette a sample or ladder replicate in any empty sample well.
- Do not touch the underside of the chip.

Agilent 2100 Bioanalyzer System

### Agilent 2100 Bioanalyzer System

• Do not touch the 2100 Bioanalyzer instrument during a run and never place it on a vibrating surface or near air-circulating instruments (for example, temperature cyclers).

#### CAUTION

The electrode cartridge may be damaged when the lid is closed.

✓ Do not force the chip to fit in the 2100 Bioanalyzer instrument.

- Clean electrodes on a daily basis using the electrode cleaner. For more details, see "Maintenance of the Electrode Cartridge" on page 110.
- Thoroughly clean electrodes on a monthly basis using a toothbrush and distilled water. For more details, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
- Clean the focusing lens once a month (or after any liquid spill) using isopropanol. For more details, see "Cleaning the Lens" on page 138.

# 2 Troubleshooting the Instrument Communication

Verify the Instrument Communication 15
Troubleshooting Communication Issues 17
Overview 17
Troubleshooting Communication Issue Flow Chart 18
Changing COM Port Settings 19
USB to Serial Adapter 20
How to create a Support Package 21

### Verify the Instrument Communication

To check whether your PC communicates with the Agilent 2100 Bioanalyzer instrument:

1 Start the instrument. The power switch is located at the rear where the power cable plugs in.

The status indicator lamp will light green if power is present and all instrument self-tests have been passed successfully.

#### NOTE

A green status indicator does not indicate that the instrument is communicating with the PC, the lamp is green even if the instrument is not connected.

- 2 Start the 2100 Expert Software.
- **3** Select the instrument tab in the **Instrument** context.
- **4** In the tree view, highlight the appropriate instrument. The connection to the selected instrument is established.
- 5 Open and close the lid the icon in the **Instrument** context should change from closed to open, see Table 1 on page 15.

#### Table 1 2100 Bioanalyzer instrument icons



Switched off or not connected to PC.



Online and lid closed.

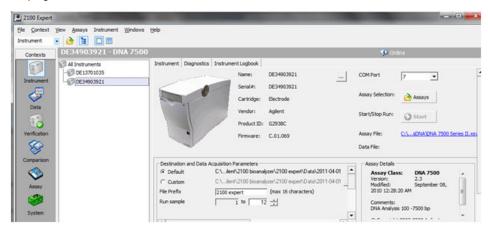


Online and lid open.

#### 2 Troubleshooting the Instrument Communication

**Verify the Instrument Communication** 

If the instrument is connected successfully, additional hardware information (serial number, cartridge type,...) is displayed on top of the screen, see Figure on page 16.



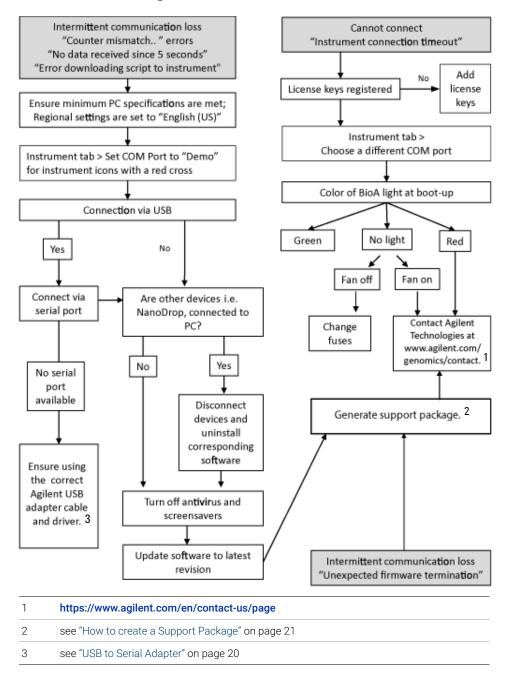
If the icon does not change, the instrument is not connected successfully. For solutions, see "Overview" on page 17.

### Troubleshooting Communication Issues

#### Overview

- Check if license keys have been registered with the software. Go to
   Help >Registration >Add Licenses. Ensure that 2 licenses have been entered:
   the instrument control license and the electrophoresis license.
- Check the COM port settings in the 2100 Expert Software, see "Changing COM Port Settings" on page 19.
- Check whether the status indicator is red. If it is red, turn off power to the 2100 Bioanalyzer instrument and turn on again. If the problem persists, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.
- Check whether the status indicator is on. If it is off and the fan is not running, replace the fuses as described under "Changing the Fuses" on page 139. A set of spare fuses comes with the instrument. If the status indicator is off and the fan is running, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a>.
- Check that the RS232 communication cable is connected as described in the Installation and Safety Guide.
- Check if another hardware device is connected to your computer.
- Replace the RS232 cable.
- Reinstall the 2100 Expert Software.
- If the 2100 Bioanalyzer instrument still will not communicate, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.

### **Troubleshooting Communication Issue Flow Chart**



**Changing COM Port Settings** 

### Changing COM Port Settings

The 2100 Bioanalyzer instrument communicates via a serial RS-232 cable with the PC. The number of COM ports available depends on the type of PC used. Laptop PCs have only one COM port. The 2100 Expert Software allows adjustment of the COM port.

To change the COM port settings:

- 1 Select the **Instrument** tab in the **Instrument** context. In the tree view, highlight the appropriate instrument.
- 2 Under **COM Port** choose a different port number from the drop down list.
- 3 Check the icon of the 2100 Bioanalyzer instrument on the screen. If it is no longer dimmed, communication between the 2100 Bioanalyzer instrument and PC is working properly. In addition, hardware information is displayed, see Figure on page 16
- 4 If you have a PC connected to your instrument and the icon is still dimmed, repeat step 2, choosing a different COM port each time, until it is not dimmed anymore. If the 2100 Bioanalyzer instrument still will not communicate create a support package ("How to create a Support Package" on page 21) and contact Agilent Technologies at
  - https://www.agilent.com/en/contact-us/page.

NOTE

The demo port refers to demo assays that do not require PC-instrument communication. For more information on demo assays, please refer to the *Online Help* or *User's Guide*.

**USB** to Serial Adapter

### USB to Serial Adapter

Agilent strongly recommends to connect the 2100 Bioanalyzer instrument directly to a serial port on the PC. However, if the PC does not offer this option, connect the 2100 Bioanalyzer instrument to USB drive utilizing an Agilent USB-Serial Adapter cable. This cable includes a controller component, which requires the installation of a driver allowing the USB to emulate a serial port.

For Expert software B.02.08 and newer

It is recommended to use the new Agilent USB-Serial Adapter cable (Part number 5188-8031, black cable) for 2100 Expert Software version B.02.08 and newer.

Please install the driver from the Agilent 2100 Expert Software UFD by clicking on the executable file found in SupportDriver/88-8031CDM21228.exe. If a UFD is not available, download the driver.

#### Install the driver

(https://www.agilent.com/cs/library/instructionsheet/public/2100%20Bioanalyzer%20Installation%20and%20Connection.zip) prior to connecting the adapter and the 2100 Bioanalyzer instrument and proceed as described below.

- Close the 2100 Expert Software.
- Execute the installation program of the driver and follow the instructions. Reboot the operating system.
- Physically connect the 2100 Bioanalyzer instrument and the laptop by using the USB-Serial Adapter cable and the standard Serial RS-232 cable.

#### 2 Troubleshooting the Instrument Communication

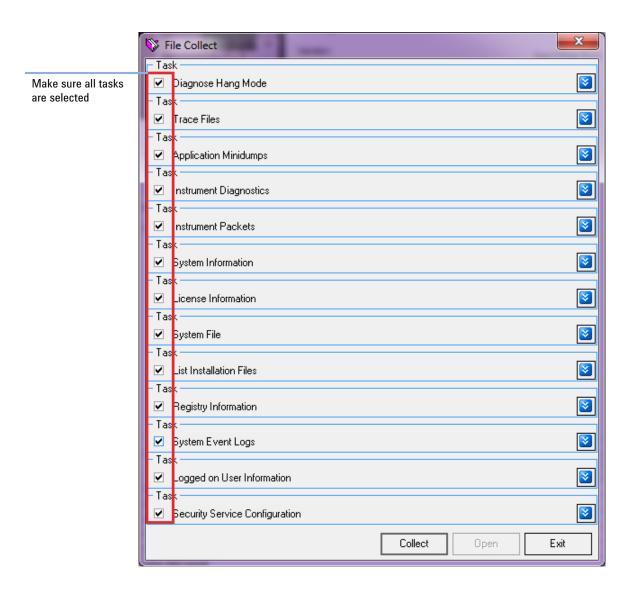
How to create a Support Package

### How to create a Support Package

The Support Package collects files and puts them into a ZIP file named similarly to *Expert\_06122015\_164231*. The ZIP file is saved automatically on your desktop and has to be send out manually (via email) to Agilent support.

In order to create a Support Package, in the 2100 Expert software, open Help > Create support package. In the pop-up window, a list of tasks is presented. Make sure all tasks are selected, then click **collect** to proceed.

How to create a Support Package



#### NOTE

#### Alternatively:

- In the Windows Start menu, open Agilent 2100 Bioanalyzer > Create support package. In the pop-up window, a list of tasks is presented.
- Make sure all tasks are selected, then click **collect** to proceed.

## 3 Troubleshooting the 2100 Expert Software

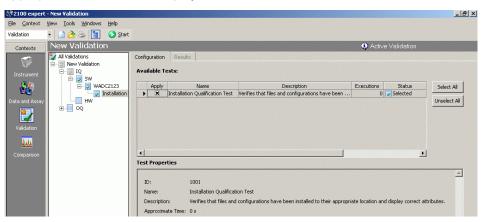
Run Installation Qualification Test 24

**Run Installation Qualification Test** 

### Run Installation Qualification Test

If it is suspected that the 2100 Expert Software is not working properly, check for corrupted or missing files.

- 1 Start the 2100 Expert Software and select the **Validation (Verification)** context.
- 2 In the tree view, select New Validation (Verification) >Installation Validation (Verification) >Software >[My PC Name] >Installation Qualification Test.
- 3 Under Available Tests select the check box of the Installation Qualification Test. This test verifies that files and configurations have been installed to their appropriate locations and display correct attributes.

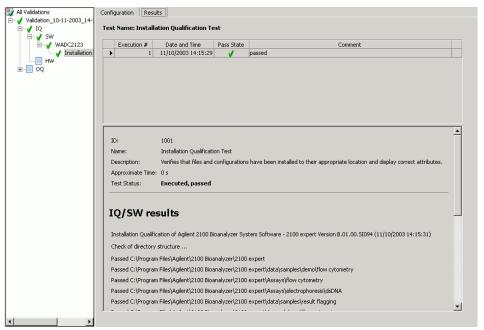


- 4 Start the software test tool by clicking **Start**.
- 5 The **Save As** dialog box appears. Define the name and location of the verification file.
- **6** When the test is finished, the **Verification Run Complete** message appears.



**Run Installation Qualification Test** 

7 The result of the installation qualification test depends on whether the software installation is complete and no files are corrupted. To review the results, switch to the **Results** tab:



- 8 If the test passes and the 2100 Bioanalyzer system still does not function properly, see "Verify the Instrument Communication" on page 15 and "Overview" on page 27 for further troubleshooting procedures. Finally, to check the application, see "Overview" on page 32, "Overview" on page 63, or "Overview" on page 84.
- **9** If the test fails, reinstall the 2100 Expert Software using the software UFD media that is supplied with the system.
- **10** If the test continues to fail, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a>.

Overview 27 Diagnostic Test Procedure 29

Overview

#### Overview

Several tests are provided for the 2100 Bioanalyzer system to check the functionality of the hardware. These tests should be performed on a regular basis, or if incorrect measurements or error messages occur. Table 2 on page 27 provides an overview of the available test chips.

Table 2 2100 Bioanalyzer System Test Chips - Electrophoresis Mode

Test chip type	Comment	Quant.
Autofocus test chip	Values for fluorescence and offset are printed on the chip; can be used multiple times.	1
Electrode/Diode test chip	Can be used multiple times.	1

Overview

Table 3 on page 28 shows a complete list of hardware diagnostic tests that can be run with the electrode cartridge.

Table 3 Diagnostic tests for electrophoresis mode

Test	Description
Electronic test	Verifies proper functioning of all electronic boards.
Fan test	Checks that the fan is running.
Lid sensor test	Checks for the devices sensing open or closed lid, and for laser and LED off when lid is closed.
Temperature test	Checks that the temperature ramp up speed of the heater plate is within specifications.
Stepper motor test	Checks for proper movement of the stepper motor.
Electrode/Diode test	Checks photodiode and current versus voltage performance of the 2100 Bioanalyzer instrument. Electrode/Diode test chip required.
High voltage stability test	Checks the accuracy and stability of all 16 high voltage power supplies. Unused chip (DNA, RNA or protein) required.
High voltage accuracy test	Checks high voltage controller. Unused chip (DNA, RNA or protein) required.
High voltage accuracy-on load test (only for G2939A, G2938B and C instruments)	Checks channel-reference diode in transmission direction. Unused chip (DNA, RNA or protein) required.
Short circuit test	Checks for instrument leak currents using an empty chip. Note: the limits of this test specify an ambient temperature of 25°C and relative humidity less than or equal to 50%. Higher temperatures of relative humidity could result in a leak current. Unused chip (DNA, RNA or protein) required.
Optics test	Checks for proper alignment of internal optics and proper function of the laser. Electrode/Diode test chip required.
Autofocus test	Checks focusing capability of optical system. Autofocus test chip required. Input values are located on top of the chip.
Laser stability test	Measures red laser signal stability. Autofocus test chip required.

**Diagnostic Test Procedure** 

### Diagnostic Test Procedure

For details on the test procedure, please refer to the documentation included with the test chip kits.

#### NOTE

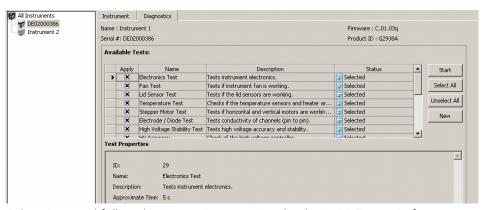
Diagnostic tests cannot be run while the 2100 Expert Software is performing a chip run.

- 1 Start the 2100 Expert Software.
- **2** Access the hardware diagnostic tests by selecting the **Diagnostics** tab in the **Instrument** context of the 2100 Expert Software.
- 3 In case two 2100 Bioanalyzer instruments are connected to the PC, highlight the appropriate instrument in the tree view.

#### NOTE

Tests can only be performed if the instrument is online. In the offline mode, the test entries are dimmed.

**4** Select any of the hardware tests from the list given or choose **Select All** to run all tests.



- **5** Select **Start** and follow the instructions as given by the 2100 Expert Software.
- **6** At the end of the procedure, all tests must have passed.

**Diagnostic Test Procedure** 

- 7 If there are failures, repeat the failed tests.
- 8 If failures persist, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a>.

The results of diagnostic tests are stored as .xdy files in Agilent\2100 bioanalyzer\2100 expert\diagnosis. If tests fail, send the .xdy files to Agilent Technical Support.

### 5 Troubleshooting the DNA Application

```
Overview
            32
Symptoms (DNA)
Residual Gel in Spin Filter after Centrifugation 34
Too High Quantitation Results 34
Too Low Quantitation Results 35
Wrong Sizing Result 36
Run Aborted 37
Chip Not Detected 38
Additional Sample or Ladder Peaks 39
Spikes 40
Low Signal Intensity 41
Missing Peaks 43
Missing Upper Marker 44
Broad Peaks 45
Baseline Dips 46
Baseline Noise 47
Baseline Jumps 48
Wavy Baseline 49
Late Migration 50
Peak Tailing 52
Unexpected Run Time 53
Error Message: No data received since 5 seconds 57
Symptoms (High Sensitivity DNA)
Artefact Peaks 59
Split Peaks 60
Baseline Negative Dips 61
```

Overview

#### Overview

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Additional information regarding the nature of a problem may be found in the **Run Log** for the data file. Select the **Log Book** tab in the **Data and Assay** context. The **Run Log** lists all the actions and errors that occurred during the run.

In rare cases, results generated by the 2100 Bioanalyzer system might not be as expected. To help find the reason for the discrepancy, see "Symptoms (DNA)" on page 33.

For most observations, there will be at least one corresponding example, depicting a typical electropherogram, gel-like image or result table. Once the observation that resembles the outcome of the experiment has been identified, a set of assigned causes will be listed by priority.

The causes are grouped into three levels:

- Most probable cause
- Probable cause
- Least probable cause

A list of solutions to help fix the problems are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.

Symptoms (DNA)

### Symptoms (DNA)

Click to go straight to the troubleshooting hints.

- "Residual Gel in Spin Filter after Centrifugation" on page 34
- "Too High Quantitation Results" on page 34
- "Too Low Quantitation Results" on page 35
- "Wrong Sizing Result" on page 36
- "Run Aborted" on page 37
- "Chip Not Detected" on page 38
- Additional Sample or Ladder Peaks" on page 39
- War "Spikes" on page 40
- Low Signal Intensity" on page 41
- Missing Peaks" on page 43
- Missing Upper Marker" on page 44
- Broad Peaks" on page 45
- Baseline Dips" on page 46
- "Baseline Noise" on page 47
- Baseline Jumps" on page 48
- Wavy Baseline" on page 49
- Late Migration" on page 50
- Peak Tailing" on page 52
- Will "Unexpected Run Time" on page 53
- Error Message: No data received since 5 seconds" on page 57

### Residual Gel in Spin Filter after Centrifugation

Most probable causes	Solution
Gel was filtered at insufficient g-value.	Refer to the Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for gel filtration.	Repeat centrifugation step at room temperature.
Gel was too cool or viscous.	Reagents must be equilibrated at room temperature for 30 minutes prior to use.

### **Too High Quantitation Results**

Most probable causes	Solution
Pipetting error during preparation of reagent mixes.	Check dilution procedure and check calibration of pipette.
Chip pipetting error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
Probable causes	Solution
Dye concentration too low (marker disappears).	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect dye from light during this time.
Low or missing upper marker.	Check "Missing Upper Marker" on page 44.
Least probable causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 minutes.

Back to "Symptoms (DNA)" on page 33

Symptoms (DNA)

### **Too Low Quantitation Results**

Most probable causes	Solution
Pipetting error during preparation of reagent mixes.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to the set-point.
Probable causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 minutes.
Dye concentration too high.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.
Least probable causes	Solution
Sample concentration too high.	Use sample concentration according to the Kit Guide.

Back to "Symptoms (DNA)" on page 33

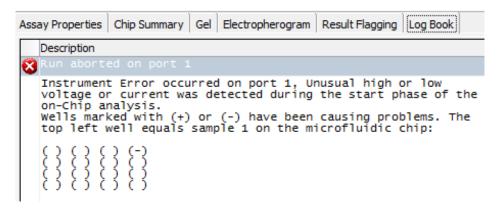
Symptoms (DNA)

### **Wrong Sizing Result**

Most probable causes	Solution
DNA ladder degraded.	Check expiration date of reagents.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket, and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Chip contaminated.	Wear powder-free gloves only. Do not touch the underside of the chip. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Markers called incorrectly.	Manually assign lower marker. Follow instructions for "Manual Marker Assignment" on page 54
Probable causes	Solution
Loaded chip kept for too long before run.	Prepared chips must be used within 5 minutes.
No ladder in ladder well.	Prepare a new chip.
Least probable causes	Solution
Vibration of 2100 Bioanalyzer instrument.	Do not touch 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Changes of ambient temperature of more than 5°C during the run.	Place 2100 Bioanalyzer instrument in thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

Back to "Symptoms (DNA)" on page 33

#### Run Aborted



#### NOTE

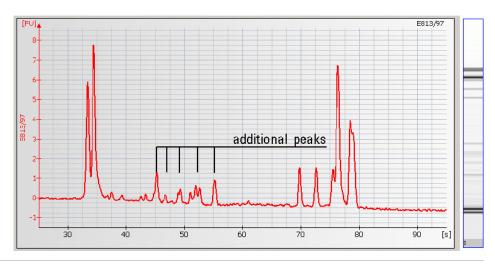
In the logbook, an error will appear: Run aborted on port x. Instrument error occurred on port x, Unusual high or low voltage or current was detected during the start phase of the on-chip analysis. The marked wells will indicate the wells on the chip that caused the problem.

Most probable causes	Solution
Insufficient volume in well(s).	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain sufficient ladder, samples or buffer.
Air bubble at the bottom of the well, obstructing access to microchannels.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of the well will not affect the assay).
Dirty electrodes.	Clean electrodes according to instructions in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket, and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes prior to use.  Store chips at room temperature.
Least probable causes	Solution
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

### **Chip Not Detected**

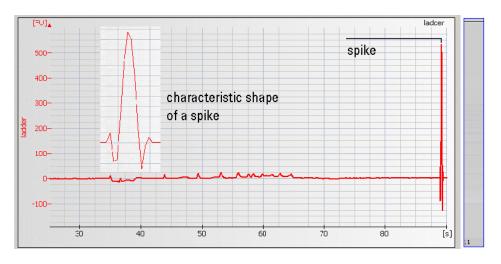
Solution
Check instrument communication as described in "Verify the Instrument Communication" on page 15.
Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain ladder, samples or buffer.
Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket, and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Solution
Prepare new chip with fresh reagents.
Solution
Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

### **Additional Sample or Ladder Peaks**



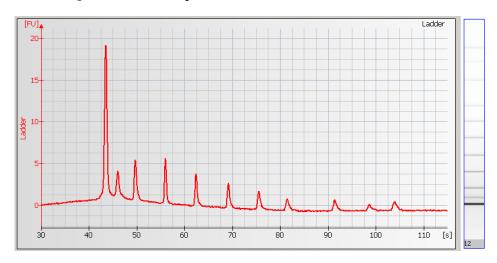
Most probable causes	Solution
Chip or gel-dye mix contaminated with particles.	Prepare new chip with new gel-dye mix: Wear powder-free gloves only. Do not touch the underside of the chip. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Sample degraded or contaminated.	Always wear gloves when handling chips and samples.
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station, see "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket, and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Vibration of 2100 Bioanalyzer instrument.	Do not touch 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the gel-dye mix should be taken up from the top of the tube.
Least probable causes	Solution
DNA ladder degraded.	Check expiration date of reagents. Use fresh DNA ladder.

### **Spikes**



Solution
Do not touch 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Prepare new chip with new gel-dye mix: Wear powder-free gloves only. Do not touch the underside of the chip. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the gel-dye mix should be taken up from the top of the tube.
Solution
Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.

# **Low Signal Intensity**



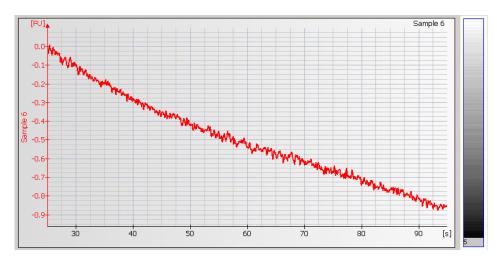
Most probable causes	Solution
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Pipetting error during preparation of reagent mixes.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Pipette new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Holding the pipette at a slight angle will ensure proper dispensing of the liquid. Use appropriate pipette and tips.
Probable causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in "Cleaning the Lens" on page 138. Do not touch the underside of the chip.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA vortexer for chip vortexing. Adjust speed to set-point.
Least probable causes	Solution

# **Troubleshooting the DNA Application** Symptoms (DNA)

5

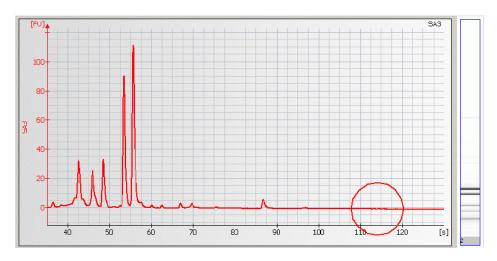
Wear powder-free gloves only.
Do not touch the underside of the chip.
Do not touch the wells of the chip.
Clean the electrodes.
Load the chip immediately after taking it out of its sealed
bag.
Do not touch 2100 Bioanalyzer instrument during a run.
Remove vibration devices, such as vortexers and vacuum
pumps, from bench.
Check autofocus using the "Hardware Diagnostics" on
page 26. If autofocus fails, contact Agilent Technologies at
https://www.agilent.com/en/contact-us/page.

### Missing Peaks



Most probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized DNase free water, if necessary.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Leak currents due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Least probable causes	Solution
Laser broken.	Perform Optics, Autofocus, and Laser Stability tests as described in "Hardware Diagnostics" on page 26. If tests fail, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .
Autofocus failure or high voltage power supply defective	Check autofocus and high voltage power supply by means of the "Hardware Diagnostics" on page 26. If a diagnostic test fails, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.

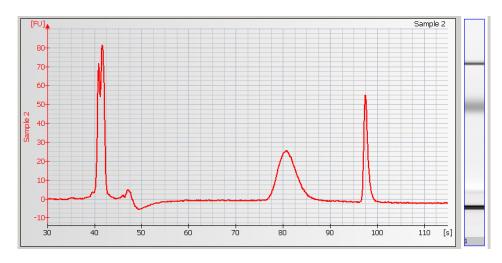
### **Missing Upper Marker**



Most probable causes	Solution
Alignment of upper marker not set properly.	Manually assign upper marker. Follow instructions for "Manual Marker Assignment" on page 54
Upper marker digested by restriction enzymes.	Inactivate restriction enzymes by adding EDTA or heat according to the manufacturer's instructions.
Probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized DNase free water if necessary.

Back to "Symptoms (DNA)" on page 33

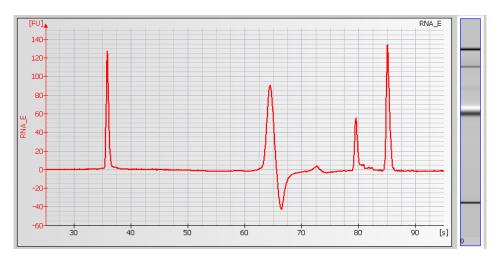
#### **Broad Peaks**



Most probable causes	Solution
Leak currents due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check the priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Dye concentration too high.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.
Probable causes	Solution
Genomic DNA or cDNA contamination.	Check DNA preparation procedure.

Back to "Symptoms (DNA)" on page 33

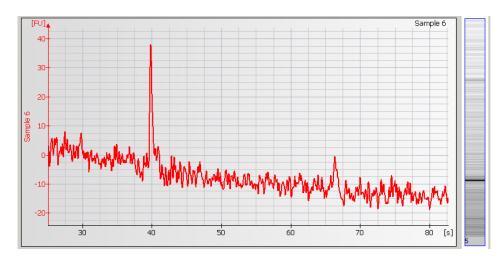
# **Baseline Dips**



Most probable causes	Solution
Sample concentration is too high.	Use sample concentration according to the Kit Guide.
Sample impurities: e.g. genomic DNA, ss DNA, etc.	Check DNA-isolation protocol. If possible, clean up samples.
Probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized DNase free water, if necessary.
Dye concentration is too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Least probable causes	Solution
Autofocus failure.	Check autofocus by means of the "Overview" on page 27. If the diagnostic test fails, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

Back to "Symptoms (DNA)" on page 33

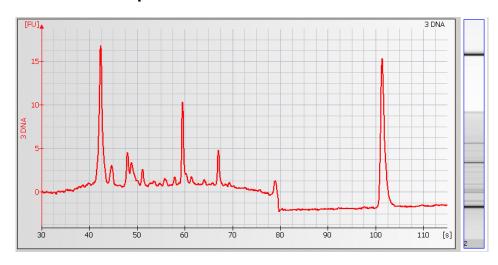
#### **Baseline Noise**



Most probable causes	Solution
Chip contaminated.	Wear powder-free gloves only. Do not touch the underside of the chip. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in "Cleaning the Lens" on page 138. Do not touch the underside of the chip.
Least probable causes	Solution
Autofocus failure or high voltage power supply defective.	Check autofocus and high voltage power supply by means of the "Hardware Diagnostics" on page 26. If tests fail, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

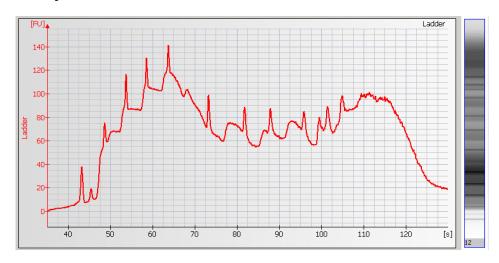
Back to "Symptoms (DNA)" on page 33

### **Baseline Jumps**



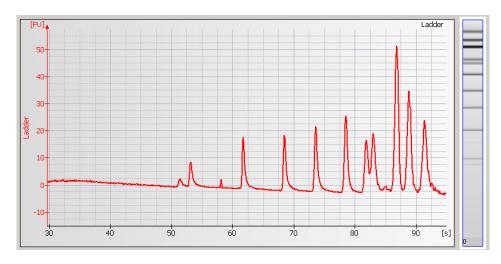
Most probable causes	Solution
Vibration of 2100 Bioanalyzer instrument.	Remove vibration devices, such as vortexers and vacuum pumps, from bench.
Instrument lid was touched during the run.	Do not touch the 2100 Bioanalyzer instrument during a run.
Least probable causes	Solution
Laser defective.	Check laser using the "Hardware Diagnostics" on page 26. If the diagnostic test fails, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.

### **Wavy Baseline**



Most probable causes	Solution
Leak currents due to contaminated electrodes.	Clean electrodes as described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Leak currents due to liquid spillage on top of the chip (detergents in sample buffer lower surface tension in the wells of the chip).	Prepare a new chip. Lower vortexing speed or mix samples manually.
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.
Probable causes	Solution
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check the priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Leak currents due to wet cartridge.	Use only 350 $\mu$ L of water in the cleaning chip. Ensure the humidity in the room is below 70% at 15 – 27 °C (59 – 81 °F).
Least probable causes	Solution
Changes in ambient temperature of more than 5°C during the run.	Place the 2100 Bioanalyzer instrument in a thermally stable environment.
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

## **Late Migration**



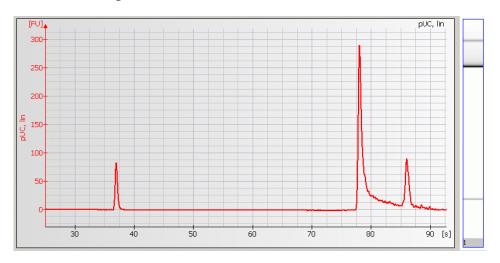
Solution
Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Clean electrodes as described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Prepare a new chip. Lower vortexing speed or mix samples manually.
Solution
Gel or gel-dye mix expired or stored incorrectly. Check Kit Guide for proper storage of gel and gel-dye mix. Use gel-dye mix within indicated time. Do not use expired reagents.
Use dye concentration according to the Kit Guide. Let the dye warm up to room
temperature for 30 minutes before preparing the gel-dye mix.
temperature for 30 minutes before preparing the gel-dye mix.  Vortex chip for 1 minute. Only use the IKA vortexer. Ensure speed is adjusted to the setpoint.

# Troubleshooting the DNA Application Symptoms (DNA)

5

Least probable causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock).  Replace vortex adapter as described in "Maintenance of the Vortexer" on page 144.
Changes in ambient temperature of more than 5°C during the run.	Place the 2100 Bioanalyzer instrument in a thermally stable environment.

### **Peak Tailing**

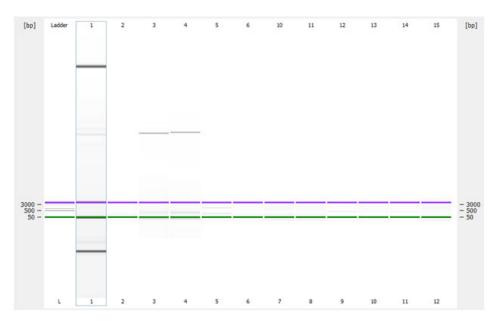


Most probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized DNase free water, if necessary.

Back to "Symptoms (DNA)" on page 33

### **Unexpected Run Time**

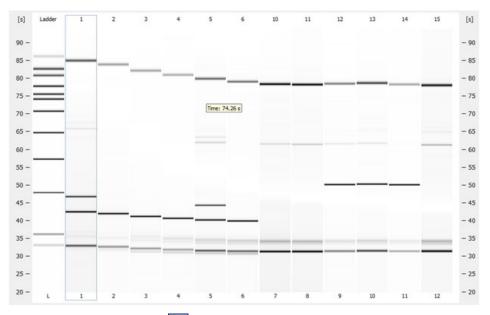
#### **Unexpected Run Time**



Most probable causes	Solution
Lower and/or upper markers are called incorrectly.	Turn off alignment and check which bands are the correct lower and upper markers. For more details, see "Manual Marker Assignment" on page 54.

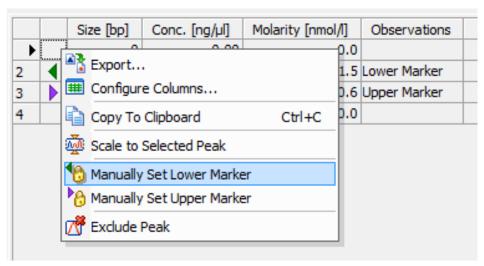
#### **Manual Marker Assignment**

1 Turn alignment off . Check the gel-like image to identify which bands are the correct lower and upper markers.

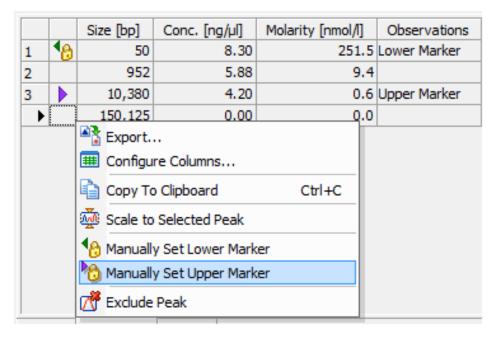


2 Turn the alignment back on . Check the electropherogram of each sample. Go to the **Peak Table** tab to adjust the markers.

**3** To adjust the lower marker, right click on the correct peak, and choose **Manually Set Lower Marker**.



4 To adjust the upper marker, right click on the correct peak, and choose Manually Set Upper Marker.

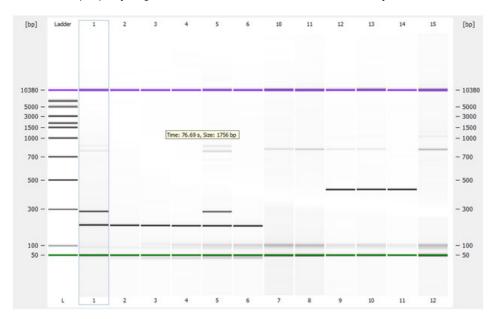


#### **Troubleshooting the DNA Application**

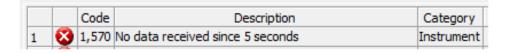
Symptoms (DNA)

5

The data is properly aligned after the markers are called correctly.



### **Error Message: No data received since 5 seconds**



Most probable causes	Solution
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 14 for troubleshooting instrument communication issue.  Ensure the Agilent USB-Serial Adapter cable, black cable (5188-8031) for 2100 Expert Software version B.02.08 and greater is used to connect the 2100 Bioanalyzer instrument to the computer through a USB port. See "USB to Serial Adapter" on page 20.

Symptoms (High Sensitivity DNA)

### Symptoms (High Sensitivity DNA)

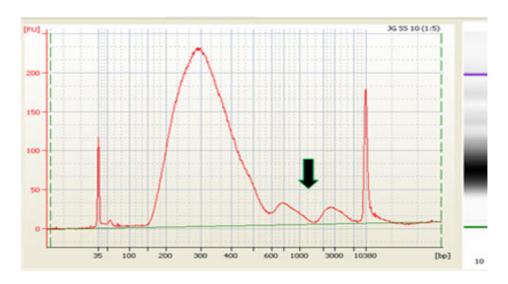
#### NOTE

Follow protocols appropriate for the Next Generation Sequencing or targeted-enrichment system used. Additional inquiries should be directed to the manufacturer of those products.

Click to go straight to the troubleshooting hints.

- Artefact Peaks" on page 59
- Split Peaks" on page 60
- Waseline Negative Dips" on page 61

#### **Artefact Peaks**

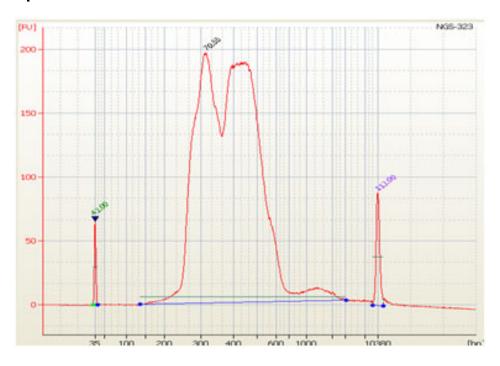


Most probable causes	Solution
Samples are in water.	Refer to the Kit Guide for sample buffer specifications. For optimal results, samples should be dissolved in 10 mM Tris and 1 mM EDTA.
Probable causes	Solution
Chip, gel-dye mix, or samples are contaminated with particles.	Wear powder-free gloves only. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.

Back to "Symptoms (High Sensitivity DNA)" on page 58

Symptoms (High Sensitivity DNA)

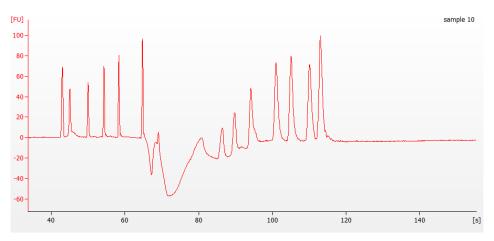
### **Split Peaks**



Most probable causes	Solution
Sample concentration is too high.	Prepare a new chip. Follow guidelines in the Kit Guide for the amount of sample to be loaded.

Back to "Symptoms (High Sensitivity DNA)" on page 58

### **Baseline Negative Dips**



Most probable causes	Solution
Residual RNase ZAP and/or SDS contamination on the electrode pins.	Only use RNaseZAP when decontaminating the pins according to "How to Clean the Pin Set of the Electrode Cartridge" on page 121. Clean the pin set thoroughly with water after running Protein or RNA assays. Change the water in the electrode cleaner Chip and/or the electrode cleaner Chip itself regularly.

### **6** Troubleshooting the RNA Application

Overview 63 Symptoms (RNA) Residual Gel in Spin Filter after Centrifugation 65 Too High Quantitation Results 65 Too Low Quantitation Results 66 Chip Not Detected 67 Run Aborted 68 Additional Sample or Ladder Peaks 69 Additional Saturating Bands 70 Degraded RNA Ladder and/or Samples 71 Spikes 72 Low Signal Intensity 73 Baseline Noise 74 Broad Peaks 75 Missing Peaks 76 Missing RNA Fragment 77 Wavy Baseline 78 Late Migration 79 Error Message: No data received since 5 seconds 80 RNA Integrity Number (RIN): N/A 81

Overview

#### Overview

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Additional information regarding the nature of a problem may be found in the run log for the data file. Select the Log Book tab in the Data and Assay context. The Run Log lists all the actions and errors that occurred during the run.

In rare cases, results generated by the 2100 Bioanalyzer system might not be as expected. To help find the reason for the discrepancy, see "Symptoms (RNA)" on page 64.

For most observations there will be at least one corresponding example depicting a typical electropherogram, gel-like image or result table. Once the observation that resembles the outcome of the experiment has been identified, a set of assigned causes will be listed by priority.

The causes are grouped into three levels:

- Most probable cause
- Probable cause
- Least probable cause

A list of solutions to help fix the problems are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.

#### 6 Troubleshooting the RNA Application

Symptoms (RNA)

#### Symptoms (RNA)

Click to go straight to the troubleshooting hints.

- "Residual Gel in Spin Filter after Centrifugation" on page 65
- "Too High Quantitation Results" on page 65
- "Too Low Quantitation Results" on page 66
- "Chip Not Detected" on page 67
- "Run Aborted" on page 68
- Additional Sample or Ladder Peaks" on page 69
- Madditional Saturating Bands" on page 70
- Lu "Degraded RNA Ladder and/or Samples" on page 71
- Was "Spikes" on page 72
- Low Signal Intensity" on page 73
- Waseline Noise on page 74
- Broad Peaks" on page 75
- Wissing Peaks" on page 76
- Wissing RNA Fragment" on page 77
- Wavy Baseline" on page 78
- Late Migration" on page 79
- Error Message: No data received since 5 seconds" on page 80
- RNA Integrity Number (RIN): N/A" on page 81

### Residual Gel in Spin Filter after Centrifugation

Most probable causes	Solution
Gel was filtered at insufficient g-value.	Refer to the Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for gel filtration.	Repeat centrifugation step at room temperature.
Gel was too cool or viscous.	Reagents must be equilibrated at room temperature for 30 minutes prior to use.

#### **Too High Quantitation Results**

Most probable causes	Solution
Pipetting error during preparation of ladder or samples.	Check dilution procedure and calibration of pipettes.
Chip pipetting error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust speed to set-point.
Probable causes	Solution
RNA ladder degraded.	Prepare a new chip using a new ladder aliquot. Always wear gloves when handling chips and RNA samples to avoid contamination. Follow decontamination procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Electrodes contaminated with RNases.	Clean electrodes with RNaseZAP. Follow decontamination procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 min before preparing the gel-dye mix. Protect the dye from light during this time.
RNA ladder not denatured.	Heat denature the RNA ladder as described in the Kit Guide.

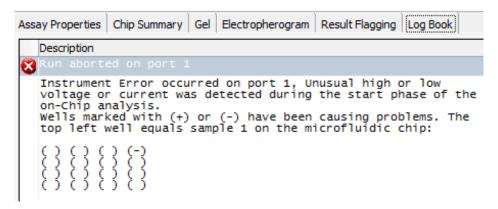
#### **Too Low Quantitation Results**

Reference measurement (e.g. UV absorption) was elevated due to contaminants in sample.  Pipetting error during preparation of ladder, samples, or reagent mixes.  Check dilution procedure.  Always insert the pipette tip to the bottom of the well when dis lnsufficient vortexing of chip.  Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the set-point.  Probable causes  Solution  RNA ladder not denatured.  Heat denature the RNA ladder as described in the Kit Guide.  Sample concentration too high.  Use the sample concentration recommended by the Kit Guide.  Due expectration too high.  Least probable causes  Solution	
absorption) was elevated due to contaminants in sample.  Pipetting error during preparation of ladder, samples, or reagent mixes.  Use appropriate calibrated pipette and tips. Check dilution procedure. Always insert the pipette tip to the bottom of the well when distributed in the like and tips. Check dilution procedure. Always insert the pipette tip to the bottom of the well when distributed in the like and tips. Check dilution procedure. Always insert the pipette tip to the bottom of the well when distributed in the like and tips. Check dilution procedure. Always insert the pipette tip to the bottom of the well when distributed in the like and tips. Solution  Probable causes  Solution  Use the sample concentration recommended by the Kit Guide.  Least probable causes  Solution	
ladder, samples, or reagent mixes.  Check dilution procedure.  Always insert the pipette tip to the bottom of the well when dis  Insufficient vortexing of chip.  Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the set-point.  Probable causes  Solution  RNA ladder not denatured.  Heat denature the RNA ladder as described in the Kit Guide.  Sample concentration too high.  Use the sample concentration recommended by the Kit Guide.  Least probable causes  Solution	
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Sample concentration too high.  Use the sample concentration recommended by the Kit Guide.  Least probable causes  Solution	
Least probable causes Solution	
	le.
Due concentration too high Leadus concentration according to the Kit Cuide Let the due	
Dye concentration too high.  Use dye concentration according to the Kit Guide. Let the dye value temperature for 30 minutes before preparing the gel-dye mix. Find the light during this time.	'

### **Chip Not Detected**

Solution
Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain ladder, samples or buffer.
Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Solution
Test the PC-instrument communication as described in "Verify the Instrument Communication" on page 15.
Solution
Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

#### **Run Aborted**

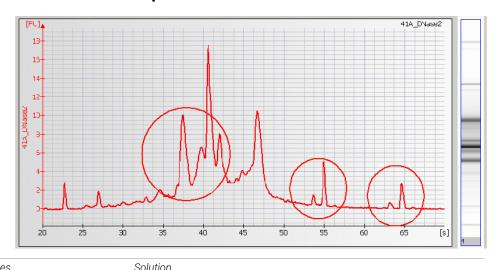


#### NOTE

In the logbook, an error will appear: Run aborted on port x. Instrument error occurred on port x, Unusual high or low voltage or current was detected during the start phase of the on-chip analysis. The marked wells will indicate the wells on the chip that caused the problem.

Most probable causes	Solution
Insufficient volume in well(s).	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain sufficient ladder, samples or buffer.
Air bubble at the bottom of the well, obstructing access to microchannels.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of the well will not affect the assay).
Dirty electrodes.	Clean electrodes according to instructions in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket, and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes prior to use.  Store chips at room temperature.
Least probable causes	Solution
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

### Additional Sample or Ladder Peaks

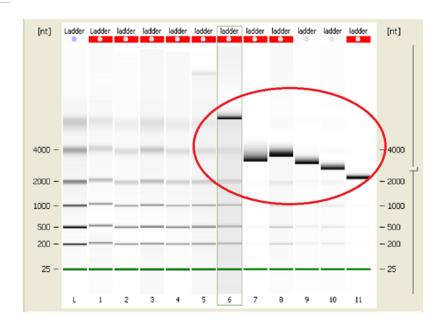


Most probable causes	Solution
Gel-dye mix expired.	Use prepared gel-dye mix within one day.
RNA ladder or sample not denatured properly.	Heat ladder or samples at 70°C for 2 minutes.
Particles in tubes.	For reagent preparation, use tubes that are supplied with the kit. Do not use autoclaved tubes.
Chip or gel-dye mix contaminated with particles.	Wear powder-free gloves only. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Probable causes	Solution
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes and vortex for 10 seconds before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the freshly prepared gel-dye mix should be taken up from the top of the tube.
Vibration of 2100 Bioanalyzer instrument	Do not touch the 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from the bench.
Chip preparation with cold reagents or chips.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use. Store chips at room temperature.
Least probable causes	Solution
RNA ladder or sample degraded.	Always wear gloves when handling chips or RNA samples to prevent them from getting contaminated. Follow decontamination procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.

#### **Additional Saturating Bands**

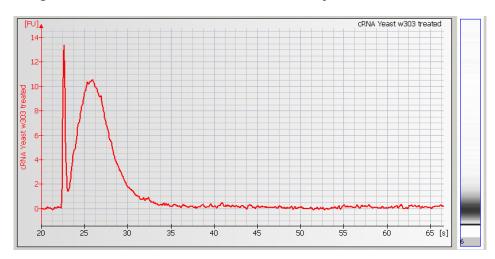
NOTE

Only present in RNA 6000 Pico or Small RNA assays.



Most probable causes	Solution
Residual RNaseZAP on electrode pins.	A dedicated electrode cassette for the use of RNA 6000 Pico and Small RNA assays. This cassette should only be washed with water in the electrode cleaner chip before and after each run. Only use RNaseZAP when decontaminating the pins according to "How to Clean the Pin Set of the Electrode Cartridge" on page 121.

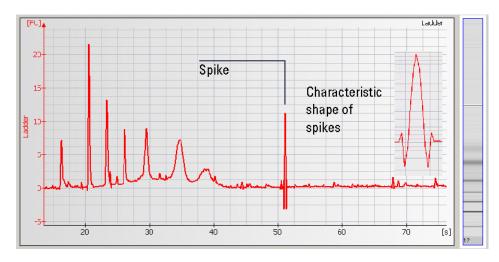
### Degraded RNA Ladder and/or Samples



Most probable causes	Solution
RNase contamination of the pin set.	Decontaminate pin set. Follow decontamination procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.  Decontaminate pipettes and work space.
RNase contamination of chips and/or reagents.	Prepare a new chip and fresh reagents. Wear powder-free gloves when preparing the chip. Decontaminate pipettes and work space.

Back to "Symptoms (RNA)" on page 64

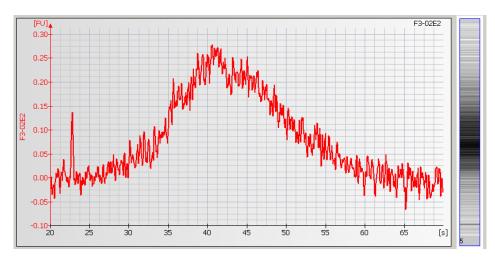
## **Spikes**



Most probable causes	Solution
Vibration of 2100 Bioanalyzer instrument.	Do not touch the 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from the bench.
Particles in tubes.	For reagent preparation, use tubes that are supplied with the kit. Do not use autoclaved tubes.
Chip or gel-dye mix contaminated with particles.	Prepare new chip with new gel-dye mix.  Wear powder-free gloves only.  Do not touch the underside of the chip.  Do not touch the wells of the chip.  Clean the electrodes.  Load the chip immediately after taking it out of its sealed bag.
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes and vortex for 10 seconds before preparing the gel-dye mix. Protect the dye from light during this time. After centrifugation, the freshly prepared gel-dye mix should be taken up from the top of the tube.
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.

Back to "Symptoms (RNA)" on page 64

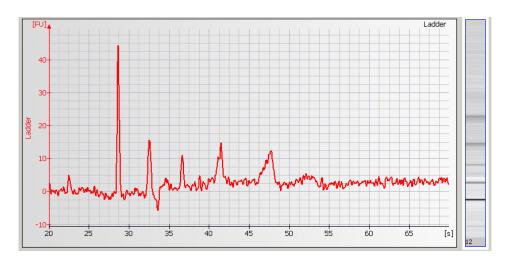
### **Low Signal Intensity**



Most probable causes	Solution
Gel-dye mix expired.	Use prepared gel-dye mix within one day.
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Pipetting error during preparation of reagent mixes.	Check dilution procedure and calibration of pipette.
Chip pipetting error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in "Cleaning the Lens" on page 138. Do not touch the underside of the chip
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use IKA vortexer. Adjust speed to set-point.
Least probable causes	Solution
Autofocus or laser failure.	Check autofocus and laser using the "Overview" on page 27. If laser stability diagnostic tests fail, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.

Back to "Symptoms (RNA)" on page 64

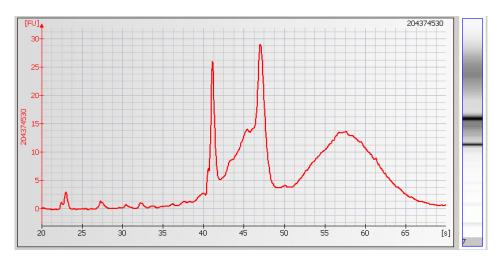
### **Baseline Noise**



Most probable causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in "Cleaning the Lens" on page 138. Do not touch the underside of the chip.
Chip contaminated with particles.	Wear powder-free gloves only. Do not touch the underside of the chip. Do not touch the wells of the chip. Clean the electrodes. Load the chip immediately after taking it out of its sealed bag.
Vibration of 2100 Bioanalyzer instrument.	Do not touch the 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from the bench.
Probable causes	Solution
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

Back to "Symptoms (RNA)" on page 64

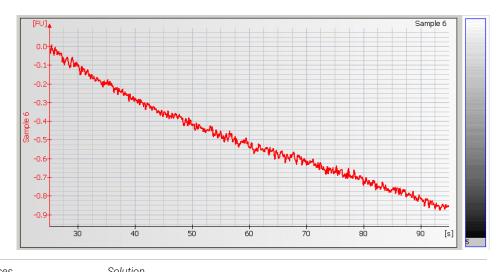
### **Broad Peaks**



Most probable causes	Solution
Sample contaminated with genomic DNA.	Check RNA isolation protocol. To remove genomic DNA, perform DNase treatment.
Leak currents due to contaminated pin set.	Clean the pin set of the electrode cartridge. Follow cleaning procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121
Probable causes	Solution
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check the priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Least probable causes	Solution
Dye concentration too high.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.

Back to "Symptoms (RNA)" on page 64.

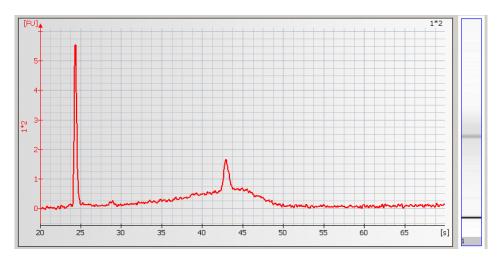
### Missing Peaks



Most probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized RNase free water, if necessary.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Leak currents due to contaminated electrodes.	Clean electrodes as described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Leak currents due to liquid spillage on top of the chip (detergents in sample buffer lower surface tension in the wells of the chip).	Prepare a new chip. Lower vortexing speed or mix samples manually.
Least probable causes	Solution
Laser broken.	Perform Laser, optics, and autofocus tests as described in "Hardware Diagnostics" on page 26. If tests fail, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.
Autofocus failure or high voltage power supply defective.	Check autofocus and high voltage power supply by means of the "Hardware Diagnostics" on page 26. If diagnostic fails, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

Back to "Symptoms (RNA)" on page 64.

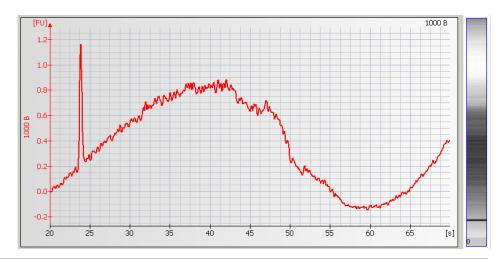
### Missing RNA Fragment



Most probable causes	Solution
Sample salt concentration is too high.	Refer to the maximum sample buffer salt limits as specified in the Kit Guide. Dilute samples with deionized RNase free water, if necessary,
Probable causes	Solution
RNase contamination of electrodes or reagents.	Clean electrodes with RNaseZAP. Follow cleaning procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.  Prepare a new chip with fresh reagents. Wear powder-free gloves when preparing the chip.

Back to "Symptoms (RNA)" on page 64.

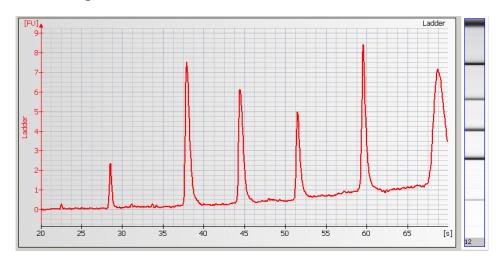
### **Wavy Baseline**



Most probable causes	Solution
Contamination with genomic DNA.	Check RNA isolation protocol. To remove genomic DNA, perform DNase treatment.
Leak currents due to contaminated electrodes.	Clean the electrode cartridge as described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121. Prepare a new chip.
Leak currents due liquid spillage on top of the chip (detergents in sample buffer lower surface tension in the wells of the chip).	Prepare a new chip. Lower vortexing speed or mix samples manually
Chip not properly primed. Clogged priming station or wrong priming station settings.	Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary.  Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Probable causes	Solution
Leak currents due to wet cartridge.	Use only 350 µL of water in the cleaning chip. Ensure the humidity in the room is below 70% at 15-27°C (59-81°F).
Least probable causes	Solution
Changes in ambient temperature of more than 5°C during the run.	Place the 2100 Bioanalyzer instrument in a thermally stable environment.
Autofocus failure or high voltage power supply defective.	Check autofocus and high voltage power using the "Overview" on page 27. If a diagnostic test fails, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

Back to "Symptoms (RNA)" on page 64

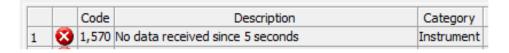
### **Late Migration**



Most probable causes	Solution
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Vortex speed too high.	Vortex chip for 1 minute. Only use the IKA vortexer. Ensure speed is adjusted to the set point.
Leak currents due to contaminated electrodes.	Clean electrodes as described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Leak currents due to liquid spillage on top of the chip (detergents in sample buffer lower surface tension in the wells of the chip).	Prepare a new chip. Lower vortexing speed or mix samples manually.
Probable causes	Solution
Vortex adapter not connected tightly.	Press vortex adapter tightly on mount (vortex adapter must not rock). Replace vortex adapter as described in "Changing the Adapter" on page 145.
Loss of gel separation properties.	Gel or gel-dye mix expired or stored incorrectly. Check Kit Guide for proper storage of gel and gel-dye mix.  Use gel-dye mix within indicated time. Do not use expired reagents.

Back to "Symptoms (RNA)" on page 64

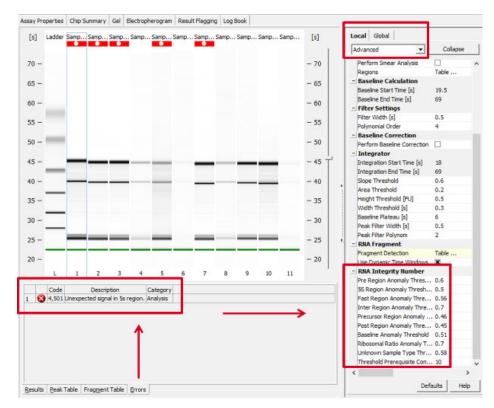
### **Error Message: No data received since 5 seconds**



Most probable causes	Solution
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 14 for troubleshooting instrument communication issue.  Ensure the Agilent USB-Serial Adapter cable, black cable (5188-8031) for 2100 Expert Software version B.02.08 and greater is used to connect the 2100 Bioanalyzer instrument to the computer through a USB port. See "USB to Serial Adapter" on page 20.

### RNA Integrity Number (RIN): N/A

Most probable causes	Solution
Incorrect Assay selected.	Check the sample type. Prepare a new Chip and re-run the samples selecting the correct Assay.
Incorrect assignment of the Lower Marker.	Please refer to Chapter "Troubleshooting the RNA Application" on page 62 to solve the problem. Prepare a new Chip and re-run the samples.
Incorrect assignment of the ribosomal bands.	Please refer to Chapter "Troubleshooting the RNA Application" on page 62 to solve the problem and re-run the samples.
Samples not heat denatured.	Heat the samples 2 min at 70 °C as described in the Kit guide. Prepare a new Chip and re-run the samples.
Un-expected signal anomalies detected in the samples (Error message <b>Unexpected signal</b> ).	Modify predefined thresholds for anomaly detection to clear the error message and get RIN displayed.



NOTE

In the **Errors** tab, an error **Unexpected signal in ...** can appear. The error message will indicate the anomaly that caused the problem. Adjust the corresponding anomaly threshold in **Advanced** mode within the RIN Setpoints group.

NOTE

RIN "N/A" is a warning that the RIN may not be reliable for a particular sample (such as due to unusual noise/signals, ribosomal ratio and other factors). Clearing the critical error message may yield a RIN, but Agilent does not guarantee the accuracy of this value. It is recommended to also perform a visual inspection of the data.

### 7 Troubleshooting the Protein Application

```
Overview
            84
Symptoms (Protein) 85
Residual Gel in Spin Filter after Centrifugation 86
Too High Quantitation Results 86
Too Low Quantitation Results 87
Wrong Sizing Result 88
Chip Not Detected 89
Run Aborted 90
Unexpected Run Time 91
Additional Sample or Ladder Peaks 95
Missing Upper Marker 96
Broad Variability of the Lower Marker 97
Missing Peaks 98
Spikes 99
Poor Reproducibility 100
Low Signal Intensity 101
Low Ladder Peaks 102
Broad Peaks 103
Baseline Dips 104
Late Migration 105
Error Message: No data received since 5 seconds 106
Symptoms (High Sensitivity Protein)
                                         107
Saturation of Lower Marker or Sample Peaks – Optical Signal too High 108
Low Signal Intensity 109
```

Overview

#### Overview

Error messages appearing on the screen describe a problem that has occurred with either the hardware or the software.

Additional information regarding the nature of a problem may be found in the Run Log for the data file. Select the **Log Book** tab in the **Data and Assay** context. The **Run Log** lists all the actions and errors that occurred during the run.

In rare cases, results generated by the 2100 Bioanalyzer system might not be as expected. To help find the reason for the discrepancy, see "Symptoms (Protein)" on page 85.

For most observations, there will be at least one corresponding example, depicting a typical electropherogram, gel-like image or result table. Once the observation that resembles the outcome of the experiment has been identified, a set of assigned causes will be listed by priority.

The causes are grouped into three levels:

- Most probable cause
- Probable cause
- Least probable cause

A list of solutions that help fix the problem are assigned to the causes. For successful troubleshooting, go through all the solution hints listed by probability.

#### 7 Troubleshooting the Protein Application

Symptoms (Protein)

### Symptoms (Protein)

Click to go straight to the troubleshooting hints.

- "Residual Gel in Spin Filter after Centrifugation" on page 86
- "Too High Quantitation Results" on page 86
- "Too Low Quantitation Results" on page 87
- "Wrong Sizing Result" on page 88
- "Chip Not Detected" on page 89
- "Run Aborted" on page 90
- Wunexpected Run Time" on page 91
- Madditional Sample or Ladder Peaks" on page 95
- Wissing Upper Marker" on page 96
- Broad Variability of the Lower Marker" on page 97
- Missing Peaks" on page 98
- Was "Spikes" on page 99
- Poor Reproducibility" on page 100
- Low Signal Intensity" on page 101
- · Low Ladder Peaks" on page 102
- Broad Peaks" on page 103
- Baseline Dips" on page 104
- Late Migration" on page 105
- Error Message: No data received since 5 seconds" on page 106

### Residual Gel in Spin Filter after Centrifugation

Most probable causes	Solution
Gel was filtered at insufficient g-value.	Refer to the Kit Guide for proper centrifuge settings.
Cooled centrifuge was used for gel filtration.	Repeat centrifugation step at room temperature.
Gel was too cool or viscous.	Reagents must be equilibrated at room temperature for 30 minutes prior to use.

### **Too High Quantitation Results**

Most probable causes	Solution
Alignment of upper marker not set properly.	Manually set upper marker. Follow instructions for "Manual Marker Assignment" on page 92.
Pipetting error during preparation of reagent mixes or chip.	Refer to the Kit Guide for proper preparation of reagents. Check dilution procedure and calibration of pipette.
Chip preparation error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable causes	Solution
Upper marker is degraded by proteases.	Treat sample with protease inhibitors prior to sample preparation.
Sample salt concentration is too high.	Check maximum sample buffer salt limits in the compatible buffer list in the Kit Guide. Dilute the sample prior to the sample preparation or use a different buffer, if possible.
Improper denaturation of sample.	Use fresh sample aliquot. Heat sample or denaturing solution for 5 minutes at 100°C. Use 0.5 mL tubes for denaturation.
Least probable causes	Solution
Loaded chip kept too long before run.	Prepared chips must be used within 5 minutes of preparation.

#### **Too Low Quantitation Results**

Most probable causes	Solution
Alignment of upper marker not set properly.	Manually set upper marker. Follow instructions for "Manual Marker Assignment" on page 92.
Pipetting error during preparation of reagent mixes or chip.	Refer to the Kit Guide for proper preparation of reagents. Check dilution procedure and calibration of pipette.
Chip preparation error.	Prepare new chip. Always insert the pipette tip to the bottom of the well when dispensing the liquid. Use appropriate pipette and tips.
Probable causes	Solution
Sample concentration too high.	Use sample concentration according to the specifications in the Kit Guides. Do not forget to dilute samples with deionized water after heat denaturation.
Diluted samples are degraded.	Use diluted samples within one day. Store samples at 4°C when not in use for longer than 1 hour.
Least probable causes	Solution
Loaded chip kept too long before run.	Prepared chips must be used within 5 minutes of preparation.
Loaded chip kept too long before run.	Prepared chips must be used within 5 minutes of prepared

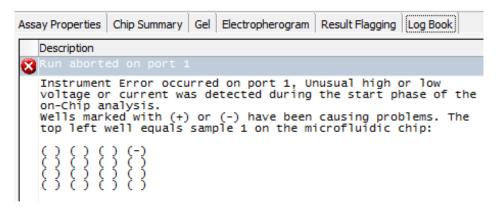
### **Wrong Sizing Result**

Most probable causes	Solution
Incorrect assignment of ladder peaks.	Check assignment of ladder peaks. For details, please refer to the Online Help o Users Guide.
Incorrect assignment of upper and/or lower marker.	Store sample buffer and denaturing solution according to the instructions given in the Kit Guide.  Check assignment of markers. Follow instructions for "Manual Marker Assignment" on page 92.
Ladder degraded.	Use diluted ladder within one day. Store ladder at 4°C when not in use for longer than 1 hour.
Probable causes	Solution
Improper denaturation of ladder.	Use fresh ladder aliquot. Heat ladder for 5 minutes at 100°C. Use 0.5 mL tubes for denaturing.
Least probable causes	Solution
Incomplete reduction of samples.	Due to disulfide bonds, some proteins will not migrate according to their molecular weight if they are not reduced properly. Proteins will migrate higher than the expected molecular weights.  Check preparation of denaturing solution described in the Kit Guide.
Protein characteristics	Glycosylation and other post-translational modifications may disturb micelle formation around the protein. The proteins will migrate higher than the expected molecular weights. This effect is reproducible.

### **Chip Not Detected**

Solution
Check Kit Guide on amount of liquid to be pipetted. Ensure all wells contain sample, ladder or buffer.
Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of well do not affect the assay).
Solution
Test the PC-instrument communication as described in "Verify the Instrument Communication" on page 15.
Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket, and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Solution
Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

#### **Run Aborted**



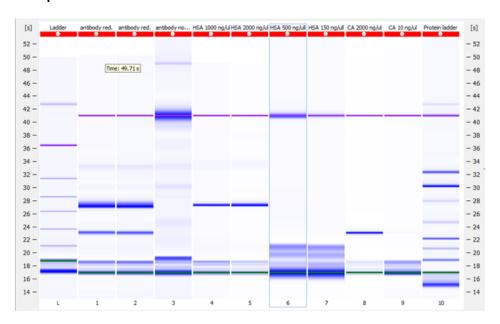
#### NOTE

In the logbook, an error will appear: Run aborted on port x. Instrument error occurred on port x, Unusual high or low voltage or current was detected during the start phase of the on-chip analysis. The marked wells will indicate the wells on the chip that caused the problem.

Most probable causes	Solution
Insufficient volume in well(s).	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain sufficient ladder, samples or buffer.
Air bubble at the bottom of the well, obstructing access to microchannels.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of the well will not affect the assay).
Dirty electrodes.	Clean electrodes according to instructions in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace syringe, gasket, and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide).
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes prior to use.  Store chips at room temperature.
Least probable causes	Solution
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 27. If the power supply is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

### **Unexpected Run Time**

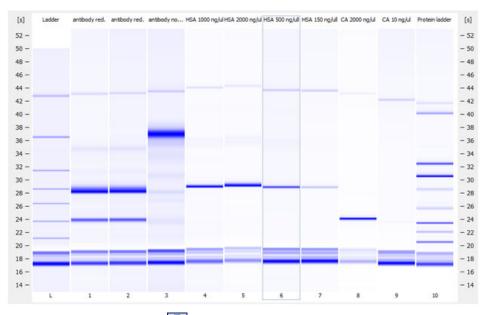
#### **Unexpected Run Time**



Most probable causes	Solution
Lower and/or upper markers are called incorrectly.	Turn off alignment and check which bands are the correct lower and upper markers. For more details see "Manual Marker Assignment" on page 92 .
Least probable causes	Solution
Bent electrode pin.	Check if electrode pins are bent or damaged. Replace electrophoresis cartridge.

#### **Manual Marker Assignment**

1 Turn alignment off . Check the gel-like image to identify which bands are the correct lower and upper markers.



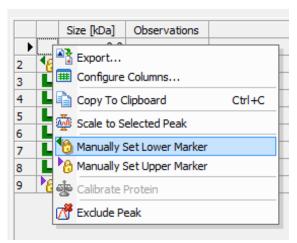
2 Turn the alignment back on . Check the electropherogram of each sample. Go to the **Peak Table** tab to adjust the markers.

#### Troubleshooting the Protein Application

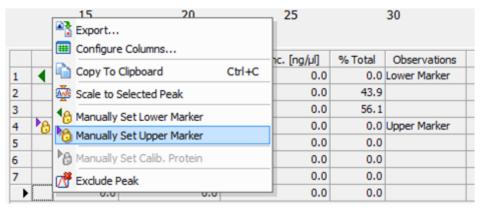
Symptoms (Protein)

7

3 To adjust the lower marker, right click on the correct peak, and choose Manually Set Lower Marker.



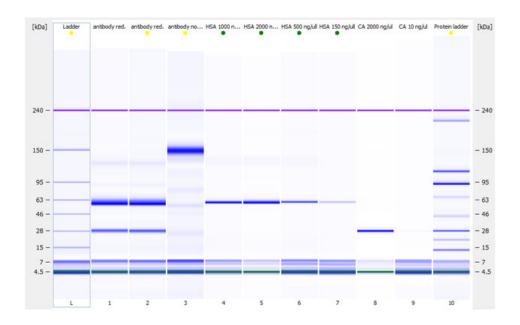
**4** To adjust the upper marker, right click on the correct peak, and choose **Manually Set Upper Marker**.



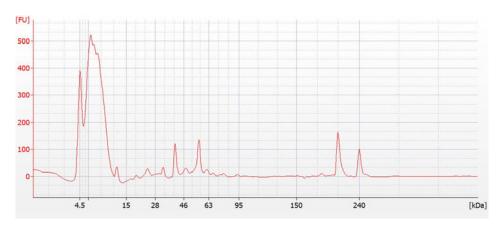
The data is properly aligned after the markers are called correctly.

## **Troubleshooting the Protein Application** Symptoms (Protein)

7

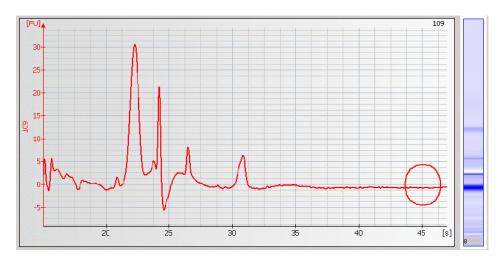


### **Additional Sample or Ladder Peaks**



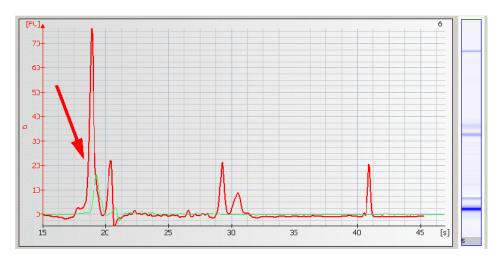
Most probable causes	Solution
Sample or ladder not denatured properly.	Prepare fresh sample aliquot. Heat sample or denaturing solution and ladder for 5 minutes at 100°C
Improper tubes used for denaturing samples.	Use 0.5 mL tubes for denaturing sample or denaturing solution.
Chip or gel-dye mix contaminated with particles.	Prepare new chip with new gel-dye mix.  Wear powder-free gloves only.  Do not touch the underside of the chip.  Do not touch the wells of the chip.  Clean the electrodes, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.  Load the chip immediately after taking it out of its sealed bag.
Probable causes	Solution
Sample degraded or contaminated.	Always wear gloves when handling chips and samples.
Ladder degraded.	Refer to the Kit Guide for proper ladder storage. Optional: Prepare ladder aliquots and use a new aliquot.
Chip prepared with cold reagents.	Prepare a new chip. Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes before use.  Store chips at room temperature.
Dye agglomerates present in the gel-dye mix.	Use dye concentration according to the Kit Guide.  Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix.  Protect the dye from light during this time.  After centrifugation, the gel-dye mix should be taken up from the top of the tube.
Vibration of 2100 Bioanalyzer instrument.	Do not touch the 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from bench.

### Missing Upper Marker



Most probable causes	Solution
Upper marker was called incorrectly.	Check upper marker assignment. Follow instructions for "Manual Marker Assignment" on page 92.
Improper preparation of sample buffer or denaturing solution.	Refer to the Kit Guide for instructions on storage and preparation of the sample buffer or denaturing solution.
Incompatible sample component. Some components of the buffer, e.g. CHAPS, TFA, etc. may interfere with the upper marker and decrease sensitivity.	See Kit Guide for a list of compatible buffers and buffer compounds. For an updated list, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> . If necessary, dilute, dialyze or desalt the sample. It is recommended to perform a serial dilution with water or compatible buffer to determine the optimal dilution.
Diluted samples are too old.	Use diluted samples within one day. Store samples at 4°C when not in use for more than 1 hour.
Probable causes	Solution
Digestion of upper marker by proteases.	Add protease inhibitor cocktails to cell lysate samples.
Improper denaturation of samples.	Use fresh sample aliquot. Heat samples with denaturing solution for 5 minutes at 100°C Use 0.5 mL tubes for denaturing samples.

### **Broad Variability of the Lower Marker**

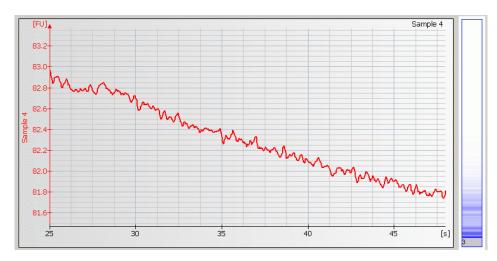


NOTE

If the lower marker is detected, the assay performance is not affected by lower marker or system peak variability.

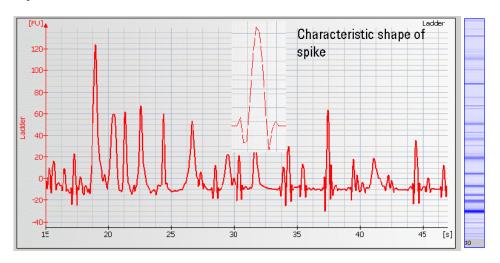
Most probable causes	Solution
Buffer components of the sample , e.g. salts, detergents, other additives etc. may interfere with the lower marker.	lonic strength of the sample buffer may affect the lower marker intensity. See Kit Guide for a list of compatible buffers and buffer compounds. For an updated list, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> . If necessary, dilute, dialyze or desalt the sample. It is recommended to perform a serial dilution with water or compatible buffer to determine the optimal dilution.

### Missing Peaks



Most probable causes	Solution
Gel-dye mix was loaded in the destain well.	Prepare a new chip.
Probable causes	Solution
Fingerprint on focusing lens or on the backside of the chip.	Clean lens as described in "Cleaning the Lens" on page 138. Do not touch the underside of the chip.
Least probable causes	Solution
Defective laser.	Check the laser stability using the "Overview" on page 27. If the laser test fails, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.
Autofocus failure.	Check autofocus using the "Overview" on page 27. If autofocus test fails, contact Agilent Technologies at https://www.agilent.com/en/contact-us/page.
High voltage power supply defective.	Check high voltage stability using the "Overview" on page 27. If the high voltage stability test fails, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

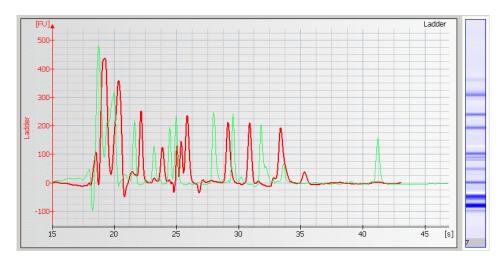
### **Spikes**



Most probable causes	Solution
Chip, gel-dye mix, destaining solution, or electrodes contaminated.	Prepare new chip with new gel-dye mix and new destaining solution.  Wear powder-free gloves only.  Do not touch the underside of the chip.  Do not touch the wells of the chip.  Clean the electrodes as described in "Maintenance of the Electrode Cartridge" on page 110.  Load the chip immediately after taking it out of its sealed bag.
Gel-dye mix or destaining solution not properly prepared.	Refer to the Kit Guide for proper preparation of the gel-dye mix and destaining solution. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Probable Causes	Solution
Vibration of 2100 Bioanalyzer instrument.	Do not touch the 2100 Bioanalyzer instrument during a run. Remove vibration devices, such as vortexers and vacuum pumps, from the bench.

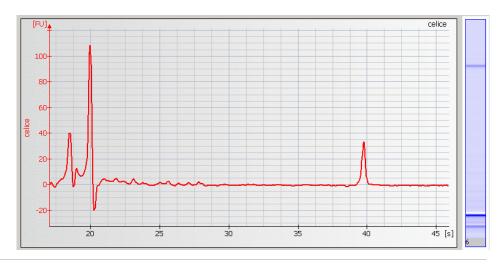
Back to "Symptoms (Protein)" on page 85

### **Poor Reproducibility**



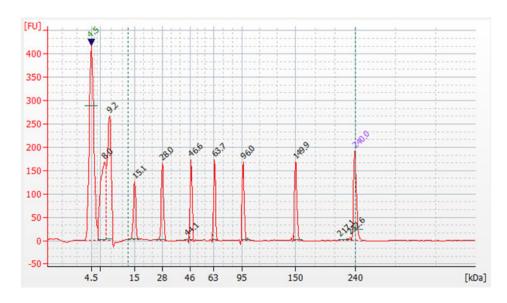
Most probable causes	Solution
Wrong peak alignment.	Check lower and upper marker assignment. Follow instructions for "Manual Marker Assignment" on page 92.
Improper denaturation of sample(s).	Use fresh sample aliquot. Heat samples with denaturing solution for 5 minutes at 100°C Use 0.5 mL tubes for denaturing samples.
Samples not prepared similarly, i.e. reducing agent (BME or DTT) was not added to all samples.	Refer to the Kit Guide for proper sample reduction.
Dirty electrodes.	Thoroughly clean the electrodes as described in "Maintenance of the Electrode Cartridge" on page 110.
Probable causes	Solution
Diluted samples are too old.	Use diluted samples within one day.
Incompatible buffer component.	See Protein Kit Guide for a list of compatible buffers and buffer compounds. For an updated list, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> . If necessary, dilute, dialyze or desalt the sample.

### **Low Signal Intensity**



Solution
Follow specifications given in the Kit Guide.
Salt concentration strongly affects the sensitivity of the assay. If necessary dilute, dialyze or desalt the sample. It is recommended to perform a serial dilution with water or compatible buffer to determine the optimal dilution.
Allow the dye to equilibrate to room temperature for 30 minutes before use. Protect dye from light during this time. Check for undissolved SDS crystals in the tube. Vortex dye well before use. If necessary, heat the sample buffer to 37°C for 2 minutes.
Dilute samples according to protocol given in the Kit Guide.
Solution
Prepare fresh sample aliquot. Heat sample and denaturating solution for 5 minutes at 100°C. Use 0.5 mL tubes for denaturation.
Check dilution procedure and check calibration of pipette(s).
Solution
Neutralize samples with appropriate buffer or dilute samples in deionized $\rm H_2O$ . Alternatively, dialyze samples against buffer with medium pH.

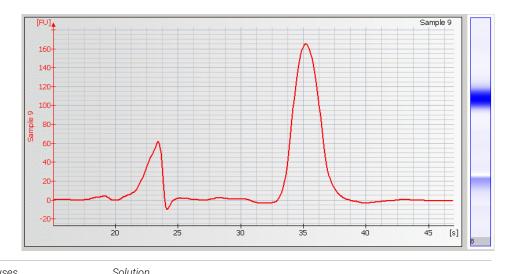
#### Low Ladder Peaks



Most probable causes	Solution
Ladder degraded.	Refer to the Kit Guide for proper ladder storage. Optional: Prepare ladder aliquots and use a new aliquot.
Ladder not diluted after denaturing.	Refer to Kit Guide for proper chip preparation.
Probable causes	Solution
Improper denaturation of ladder.	Use fresh ladder aliquot. Heat ladder for 5 minutes at 100°C. Use 0.5 mL tubes for denaturation.
Diluted ladder is too old.	Use diluted ladder within one day.
Pipetting error during preparation of reagent mixes.	Check dilution procedure and calibration of pipette.

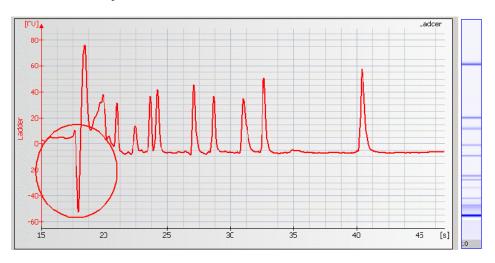
Back to "Symptoms (Protein)" on page 85

#### **Broad Peaks**



Most probable causes	Solution
Lower and/or upper markers are called incorrectly.	Turn off alignment and check which bands are the correct lower and upper markers. For more details see "Manual Marker Assignment" on page 92.
Air bubbles at the bottom of the well.	Always insert the pipette tip to the bottom of the well when dispensing the liquid. Remove large air bubbles with a pipette tip (small bubbles on top of the well will not affect the assay).
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.  Clean/replace syringe, gasket, and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide)
Leak currents due to contaminated electrodes.	Clean electrodes with analysis-grade water and a toothbrush, see "How to Clean the Pin Set of the Electrode Cartridge" on page 121.  Do not leave chip in instrument after run.  Clean electrodes with the electrode cleaner chip for 10 seconds after each run.
Probable causes	Solution
Sample was not denatured properly.	Use fresh sample aliquot. Heat sample and denaturing solution for 5 minutes at 100°C.
Samples not prepared similarly, i.e. reducing agent (BME or DTT) was not added to all samples.	Refer to the Kit Guide for proper sample reduction.

### **Baseline Dips**

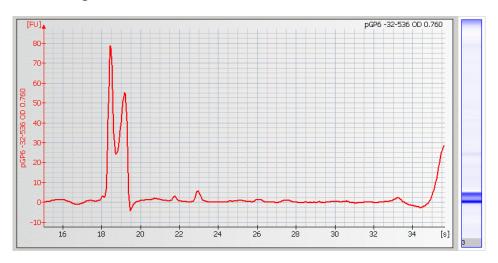


#### NOTE

If the lower marker is detected, the assay performance is not affected by dips.

Most probable causes	Solution	
Sample contains additional detergents and dyes.	See Kit Guide for a list of compatible buffers and buffer compounds. For an updated list, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> . If necessary, dilute, dialyze or desalt the sample.	
Gel-dye mix or destaining solution not properly prepared.	Refer to the Kit Guide for proper preparation of the gel-dye mix and destaining solution. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.	

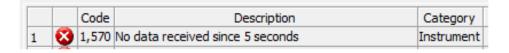
### **Late Migration**



Most probable causes	Solution
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134. Clean/replace gasket, syringe and plastic adapter, if necessary. Check if clip and base plate of priming station are in the correct position (see Kit Guide)
Protein chips expired.	Check expiration date on chip box.
Protein concentration in samples too high.	Use protein concentration according to specifications given in the Kit Guide.
Least probable causes	Solution
Defective heater plate.	Run the temperature test by using the "Overview" on page 27. If the heater plate is defective, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a> .

Back to "Symptoms (Protein)" on page 85

### Error Message: No data received since 5 seconds



Most probable causes	Solution
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 14 for troubleshooting instrument communication issue.  Ensure the Agilent USB-Serial Adapter cable, black cable (5188-8031) for 2100 Expert Software version B.02.08 and greater is used to connect the 2100 Bioanalyzer instrument to the computer through a USB port. See "USB to Serial Adapter" on page 20.

#### 7 Troubleshooting the Protein Application

Symptoms (High Sensitivity Protein)

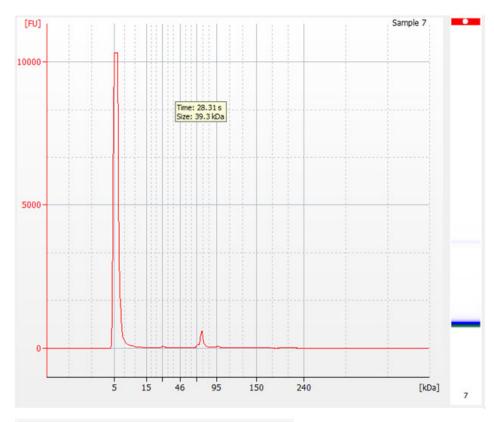
### Symptoms (High Sensitivity Protein)

Click to go straight to the troubleshooting hints.

- Katuration of Lower Marker or Sample Peaks Optical Signal too High" on page 108
- Low Signal Intensity" on page 109

Symptoms (High Sensitivity Protein)

# Saturation of Lower Marker or Sample Peaks – Optical Signal too High



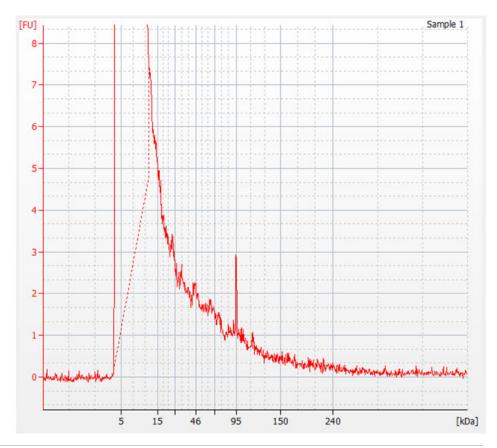
		Code	Description	Category
1	<b>3</b>	559	Optical signal too high (1605h)	Instrument

Most probable causes	Solution
Insufficient dilution of ladder or samples.	Follow instructions in the Kit Guide. Dilution of the labeling reaction by 1:200 is recommended.
Probable causes	Solution
Chip prepared with cold reagents.	Prepare a new chip.  Allow all reagents and reagent mixes to warm up to room temperature for 30 minutes prior to use.  Store chips at room temperature.

Back to "Symptoms (High Sensitivity Protein)" on page 107

Symptoms (High Sensitivity Protein)

# **Low Signal Intensity**



Most probable causes	Solution
Insufficient labeling of ladder or samples.	Follow instructions in the Kit Guide. Labeling occurs between pH 8-9. Proteins must contain lysines for labeling reaction. Check buffer compatibility in the "Compatibility List for the Labeling Reaction" in the Kit Guide.
Insufficient sample present.	Follow instructions for protein concentration in the Kit Guide. For details, see https://www.agilent.com/cs/library/technicaloverviews/public/5989-8940EN.pdf

Back to "Symptoms (High Sensitivity Protein)" on page 107

# 8 Maintenance of the Electrode Cartridge

Overview 111
DNA and Protein Assays 112
Cleaning the Electrodes after each DNA and Protein Assays 113
RNA Nano Assay 114
Cleaning the Electrodes before each RNA Nano Assay 115 Cleaning the Electrodes after each RNA Nano Assay 116 RNase Decontamination of the Pin Set 117
RNA Pico or Small RNA Assay 118
Cleaning the Electrodes before each RNA Pico or Small RNA Assay 119 Cleaning the Electrodes after each RNA Pico or Small RNA Assay 120 RNase Decontamination of the Pin Set 120
How to Clean the Pin Set of the Flectrode Cartridge 121

Overview

## Overview

The cleaning procedure of the electrode cartridge depends on the assay that is run on the 2100 Bioanalyzer system. For details see "How to Clean the Pin Set of the Electrode Cartridge" on page 121. Table 4 on page 111 gives an overview on the different cleaning procedures.

Table 4 Maintenance of the Electrode Cartridge

Assay Before each run		After each run	Monthly or after liquid spill: pin set cleaning	
DNA and Protein	Optional: Electrode cleaner: deionized H <sub>2</sub> O for 10 seconds.	Electrode cleaner: deionized $\rm H_2O$ for 10 seconds.	With brush: deionized $\rm H_2O$ or isopropanol.	
RNA Nano	<ul> <li>Electrode cleaner:</li> <li>RNaseZAP for 60 seconds.</li> <li>RNase free H<sub>2</sub>O for 10 seconds.</li> </ul>	<ul> <li>Electrode cleaner:</li> <li>RNase free H<sub>2</sub>O for 10 seconds.</li> </ul>	RNase decontamination with brush:  RNaseZAP.  RNase free H <sub>2</sub> O.	
RNA Pico and Small RNA	Electrode cleaner: • RNase free H <sub>2</sub> O for 5 minutes.	Electrode cleaner: • RNase free H <sub>2</sub> O for 30 seconds.	RNase decontamination with brush:  RNaseZAP  RNase free H <sub>2</sub> O	

NOTE Electrode cleaner should be filled with 350  $\mu$ L of solution (H<sub>2</sub>0 or RNaseZAP).

ROTE Electrode Pinset must be completely dry before adding a chip and initiating a new run.

**DNA and Protein Assays** 

# DNA and Protein Assays

Assay	Before each run	After each run	Monthly or after liquid spill: Pin set cleaning	
DNA and Protein	Optional: Electrode cleaner: deionized H <sub>2</sub> O for 10 seconds.	Electrode cleaner: deionized H <sub>2</sub> O for 10 seconds.	With brush: • deionized H <sub>2</sub> O or isopropanol	

**DNA and Protein Assays** 

# Cleaning the Electrodes after each DNA and Protein Assays

When the assay run is complete, immediately remove the used chip out of the 2100 Bioanalyzer instrument and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean and no residues are left over from the previous assay.

#### When

After each DNA and Protein run.

#### Parts required

#	p/n	Description
1	NA	Electrode cleaner (required amount included in the kits)
1	NA	Deionized analysis-grade water

### CAUTION

#### Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

- ✓ Never fill more than 350 µL of water in the electrode cleaner.
- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ L deionized analysis-grade water.
- 2 Open the lid and place electrode cleaner in the 2100 Bioanalyzer instrument.
- 3 Close the lid and leave it closed for about 10 seconds.
- **4** Open the lid and remove the electrode cleaner.
- **5** Wait another 10 seconds for the water on the electrodes to evaporate.
- **6** Empty the electrode cleaner after every cleaning procedure and refill the electrode cleaner.
- 7 After 25 Chip runs, replace the used electrode cleaner with a new one.

### NOTE

When switching between different assays, a more thorough cleaning may be required. For details, refer to "How to Clean the Pin Set of the Electrode Cartridge" on page 121.

# RNA Nano Assay

Assay	Before each run	After each run	Monthly or after liquid spill: Pin set cleaning
RNA Nano	<ul> <li>Electrode cleaner:</li> <li>RNase ZAP for 60 seconds.</li> <li>RNase free H<sub>2</sub>O for 10 seconds.</li> </ul>	<ul> <li>Electrode cleaner:</li> <li>RNase free H<sub>2</sub>O for 10 seconds.</li> </ul>	RNase decontamination with brush: RNase ZAP. RNase free H <sub>2</sub> O.

# Cleaning the Electrodes before each RNA Nano Assay

To avoid decomposition of the RNA sample, follow this electrode decontamination procedure on a daily basis before running any RNA Nano assay.

#### When

Before each RNA Nano run.

### Parts required

#	p/n	Description
2	NA	Electrode cleaner (required amount included in the kits)
1	NA	RNase-free water
1	NA	RNaseZAP (Ambion, Inc cat. no. 9780)

### NOTE

Perform the following RNase decontamination procedure on a daily basis before running any RNA Nano assays.



#### Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

✓ Never fill more than 350 µL of water in the electrode cleaner.

- 1 Slowly fill one of the wells of an electrode cleaner with 350 µL RNaseZAP.
- 2 Open the lid and place electrode cleaner in the 2100 Bioanalyzer instrument.
- 3 Close the lid and leave it closed for about 1 minute.
- 4 Open the lid and remove the electrode cleaner label the electrode cleaner and keep for future use. You can reuse the electrode cleaner for all chips in one kit.
- 5 Slowly fill one of the wells of another electrode cleaner with 350  $\mu$ L RNase-free water.
- **6** Place electrode cleaner chip in the 2100 Bioanalyzer instrument.
- 7 Close the lid and leave it closed for about 10 seconds.
- **8** Open the lid and remove the electrode cleaner. Label it and keep it for further use.
- **9** Wait another 10 seconds for the water on the electrodes to evaporate before closing the lid.



Remove the RNaseZAP and the RNase-free water from the electrode cleaner at the end of the day.

# Cleaning the Electrodes after each RNA Nano Assay

When the assay is complete, immediately remove the used chip from the 2100 Bioanalyzer instrument and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean and no residues are left over from the previous assay.

#### When

After each RNA Nano run.

### Parts required

#	p/n	Description
1	NA	Electrode cleaner (required amount included in the kits)
1	NA	RNase-free water

### NOTE

Use a new electrode cleaner with each new kit.

## CAUTION

Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

- ✓ Never fill more than 350 µL of water in the electrode cleaner.
- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ L RNase free water.
- **2** Open the lid and place electrode cleaner in the 2100 Bioanalyzer instrument.
- **3** Close the lid and leave it closed for about 10 seconds.
- 4 Open the lid and remove the electrode cleaner.
- **5** Wait another 10 seconds for the water on the electrodes to evaporate.

## NOTE

Remove the RNase-free water from the electrode cleaner at the end of the day.

## RNase Decontamination of the Pin Set

When the pin set of the electrode cartridge is suspected to be contaminated with RNases follow the instructions described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.

RNA Pico or Small RNA Assay

# RNA Pico or Small RNA Assay

Assay	Before each run After each run		Monthly or after liquid spill: pin set cleaning	
RNA Pico or Small RNA	<ul><li>Electrode cleaner:</li><li>RNase free H<sub>2</sub>O for 5 minutes.</li></ul>	<ul> <li>Electrode cleaner:</li> <li>RNase free H<sub>2</sub>O for 30 seconds.</li> </ul>	RNase decontamination with brush:  RNaseZAP  RNase free H <sub>2</sub> O	

RNA Pico or Small RNA Assay

# Cleaning the Electrodes before each RNA Pico or Small RNA Assay

To avoid decomposition of the RNA sample, follow this electrode decontamination procedure on a daily basis before running any RNA Pico or Small RNA assay.

#### When

Before each RNA Pico or Small RNA run.

#### Parts required

#	p/n	Description
1	NA	Electrode cleaner (required amount included in the kits)
1	NA	RNase-free water

Description

### NOTE

To prevent contamination problems, it is strongly recommended to use a dedicated electrode cartridge for RNA Pico and Small RNA assays.



#### Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

- ✓ Never fill more than 350 µL of water in the electrode cleaner.
- 1 Slowly fill one of the wells of an electrode cleaner with 350 µL RNase-free water.
- 2 Open the lid and place electrode cleaner in the 2100 Bioanalyzer instrument.
- 3 Close the lid and leave it closed for 5 minutes.
- **4** Open the lid and remove the electrode cleaner. Label the electrode cleaner and keep for future use.
- **5** Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.

RNA Pico or Small RNA Assay

# Cleaning the Electrodes after each RNA Pico or Small RNA Assay

When the assay is complete, immediately remove the used chip out of the 2100 Bioanalyzer instrument and dispose of it according to good laboratory practice. Then perform the following procedure to ensure that the electrodes are clean and no residues are leftover from the previous assay.

#### When

After each RNA Pico or Small RNA run.

#### Parts required

#	p/n	Description
1	NA	Electrode cleaner (required amount included in the kits)
1	NA	RNase-free water

### CAUTION

#### Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

✓ Never fill more than 350 µL of water in the electrode cleaner.

- 1 Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ L RNase-free water.
- 2 Open the lid and place electrode cleaner in the 2100 Bioanalyzer instrument.
- 3 Close the lid and leave it closed for 30 seconds.
- **4** Open the lid and remove the electrode cleaner.
- **5** Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.



Replace the water in the electrode cleaner after *each* use. Use a new electrode cleaner after 12-13 electrode cleaning procedures and with each new kit.

### RNase Decontamination of the Pin Set

When the pin set of the electrode cartridge is suspected to be contaminated with RNases follow the instructions described in "How to Clean the Pin Set of the Electrode Cartridge" on page 121.

# How to Clean the Pin Set of the Electrode Cartridge

The electrode cartridge, which includes the pin set, can be removed for cleaning.

#### When

- · On a monthly basis.
- Whenever the pin set is contaminated with liquid spill or salt deposition.
- · When the pin set is contaminated with RNases.

Tools required	p/n		Description
	NA		Compressed oil-free air
OR	NA		Desiccator
	NA		Beaker
	NA		Soft brush
Parts required	#	p/n	Description
	1	NA	Deionized analysis-grade water
	1	NA	RNase-free water
	1	NA	Unused chip to run the short circuit diagnostic test.

## CAUTION

Damage of electrodes and high voltage power supply.

- Do not touch the electrodes while the cartridge is in the 2100 Bioanalyzer instrument, this could damage the electrodes and high voltage power supply.
- 1 Turn off line power to the 2100 Bioanalyzer instrument. The line switch is located at the rear of the instrument.

2 Open the lid and pull the metal lever on the inside left of the lid to the vertical position as shown in Figure 1 on page 122. When the lever is in the vertical position, the cartridge is released from the lid by about 10 mm.

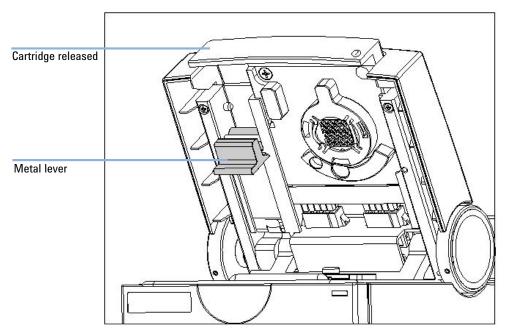


Figure 1 Remove/replace the electrode cartridge

**3** Gently pull the cartridge out of the lid as shown in Figure 1 on page 122.

**4** Open the bayonet socket of the pin set by turning the plastic lever to the left, see Figure 2 on page 123.

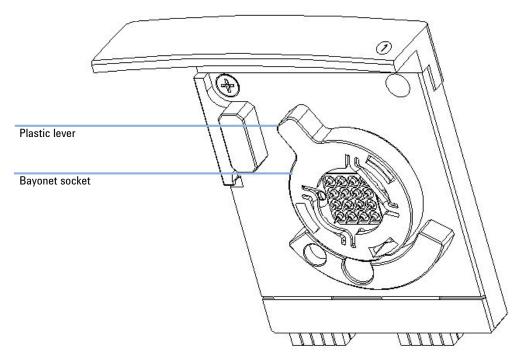


Figure 2 Bayonet socket of the electrode pin set

**5** Remove the cover of the bayonet socket by gently pulling the plastic lever. The pin set may stick to the electrode base. Remove it by carefully pulling it off, see Figure 3 on page 124.

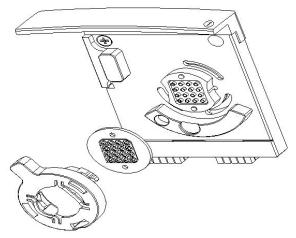


Figure 3 Bayonet cover and pin set

**6** Gently brush the pin set with a soft brush in deionized analysis-grade water or isopropanol. In case of RNase contamination, use RNaseZap (Ambion, Inc. cat. no. 9780).

## **CAUTION**

### Damage of pin set

Bending or misaligning the pins will lead to poor quality results or prematurely terminated assay runs.

- Be careful not to bend or misalign the pins.
- 7 In case of highly contaminated or dirty pins, the pin set may be autoclaved or sonicated. For autoclaving or sonicating the pin set, follow standard procedures for plastic material.
- **8** Rinse pin set thoroughly with deionized analysis-grade water when running DNA or Protein assays, or RNase-free water when running RNA assays.

## **CAUTION**

### Damp pin set

Make sure that the pin set is fully dry before placing it back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

- **9** Let the pin set completely dry in a desiccator overnight or use oil-free compressed air.
- **10** Place the pin set on the cartridge base and the bayonet cover over the pin set, see Figure 3 on page 124.
- **11** Lock the pin set to the electrode base by turning the plastic lever of the bayonet cover to the right, see Figure 2 on page 123.
- **12** Slide the electrode cartridge with the pin set into the 2100 Bioanalyzer instrument lid as shown in Figure 1 on page 122 and move the metal lever to the flat (closed) position.
- **13** Push the metal front of the electrode cartridge to ensure a tight connection to the 2100 Bioanalyzer instrument, see Figure 1 on page 122.
- **14** To verify that the electrodes are completely dry, perform the Short circuit diagnostic test from the **Diagnostics** tab in the **Instrument** context. This test takes approximately three minutes.

### CAUTION

# Damage of electrode cartridge

Heat can permanently damage the electrode cartridge.

- Do not dry the electrode cartridge in an oven.
- **15** If the short circuit test fails, the electrode assembly may still be wet. Take the pin set out of the instrument, dry it with oil-free compressed air, then repeat the test

# 9 Maintenance of the Chip Priming Station

Overview 127

Replacing the Syringe 128

Cleaning the Syringe Adapter 129

Replacing the Syringe Adapter 131

Replacing the Gasket 132

Checking the Chip Priming Station for Proper Performance - Seal Test 134

Overview

# Overview

Regular cleaning procedures are necessary to maintain the performance of the chip priming station. The table below gives an overview on the different maintenance procedures.

Procedure	Time interval	Or if
Replacing the syringe	With each new kit Latest every 3 months	syringe is broken, see "Replacing the Syringe" on page 128.
Cleaning the syringe adapter	Every 3 months	
Replacing the syringe adapter		adapter is clogged with dried gel or damaged, see "Replacing the Syringe Adapter" on page 131.
Replacing the gasket	Every 3 months	gasket is damaged, torn or contaminated with dried gel, see "Replacing the Gasket" on page 132.
Checking the chip priming station for proper seal	Every 4 weeks	gasket, syringe adapter or syringe was replaced, see "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 134.

### 9 Maintenance of the Chip Priming Station

Replacing the Syringe

# Replacing the Syringe

### **When** Quarterly or whenever it is clogged.

### Parts required

#	p/n	Description
1	NA	Syringe kit that comes with each DNA, RNA and Protein kit
1	NA	Deionized water

- 1 Unscrew the old syringe from the top of the chip priming station.
- **2** Remove clip from the old syringe. Dispose syringe according to good laboratory practices.
- **3** Slide new syringe into the clip. Ensure syringe and clip are flushed together.
- **4** Screw the syringe tight into the luer lock adapter.
- **5** Check the priming station as described in "Checking the Chip Priming Station for Proper Performance Seal Test" on page 134.

# Cleaning the Syringe Adapter

**When** Quarterly or whenever it is clogged.

Parts required	#	p/n	Description
	1	NA	Syringe kit that comes with each DNA, RNA and Protein kit
	1	NA	Deionized water

- 1 Open the priming station.
- 2 Move the mounting ring holding the adapter in place to the left as shown in Figure 4 on page 129. The ring will come off.

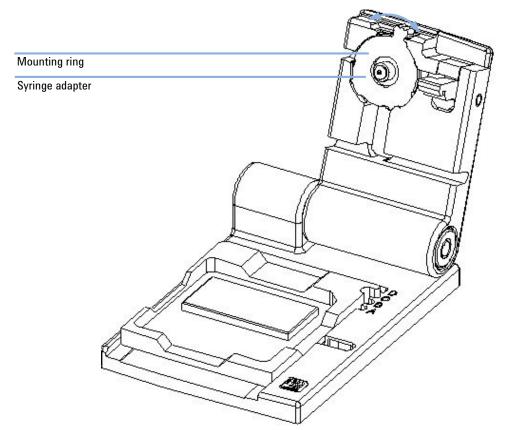


Figure 4 Mounting ring of the syringe adapter

**3** Press the syringe adapter out of its mount as shown in Figure 5 on page 130.

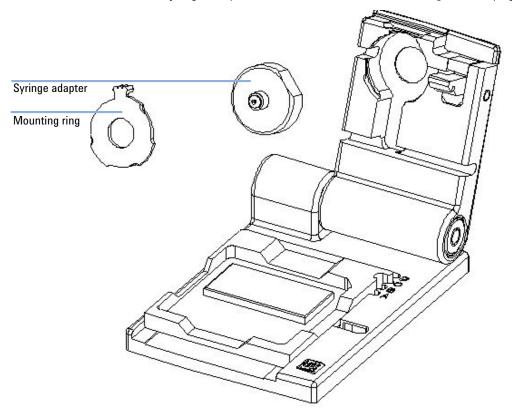


Figure 5 Removing/replacing the syringe adapter

- **4** Remove dried gel at the opening of the adapter with a needle.
- **5** Screw on syringe and flush water through the adapter several times.
- **6** Flush syringe with isopropanol.
- 7 Allow adapter to dry fully.
- **8** Insert the syringe adapter, see Figure 5 on page 130.
- **9** Follow the steps as described in "Cleaning the Syringe Adapter" on page 129 to reassemble the priming station.
- **10** Close the chip priming station.
- 11 Screw a dry syringe tight into the luer lock adapter.
- **12** Check the priming station as described in "Checking the Chip Priming Station for Proper Performance Seal Test" on page 134.

### 9 Maintenance of the Chip Priming Station

Replacing the Syringe Adapter

# Replacing the Syringe Adapter

When

If significantly clogged and unable to clean thoroughly.

### Parts required

#	p/n	Description
1	G2938-68716	Gasket kit

- 1 Follow the steps described in "Cleaning the Syringe Adapter" on page 129 to remove the syringe adapter.
- **2** Dispose the old syringe adapter.
- **3** Insert the syringe adapter, see Figure 5 on page 130.
- **4** Follow the steps as described in "Cleaning the Syringe Adapter" on page 129 to reassemble the priming station.
- **5** Check the priming station as described in "Checking the Chip Priming Station for Proper Performance Seal Test" on page 134.

Replacing the Gasket

# Replacing the Gasket

The silicone gasket, see Figure 6 on page 132, ensures a tight connetion between the chip and syringe adapter.

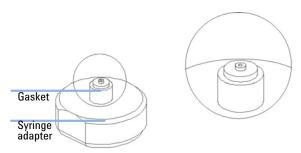


Figure 6 Syringe adapter with gasket

#### When

Quarterly or when it is torn.

### Parts required

#	p/n	Description
1	G2938-68716	Gasket kit

- 1 Remove the syringe adapter out of the chip priming station as described in "Replacing the Syringe Adapter" on page 131.
- 2 Pull out the old silicone gasket with your fingers or tweezers. See Figure 7 on page 132 for a disassembled adapter.

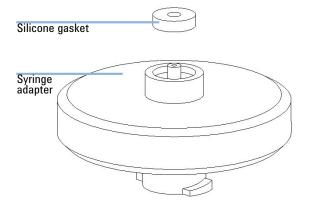


Figure 7 Syringe adapter with disassembled gasket

### 9 Maintenance of the Chip Priming Station

Replacing the Gasket

- 3 Insert a new silicone gasket and gently push into place.
- 4 Insert the syringe adapter into the chip priming station as described in "Replacing the Syringe Adapter" on page 131 and reassemble the priming station.
- **5** Check the priming station as described in "Checking the Chip Priming Station for Proper Performance Seal Test" on page 134.

Checking the Chip Priming Station for Proper Performance - Seal Test

# Checking the Chip Priming Station for Proper Performance - Seal Test

#### When

Every month or whenever a component of the priming station (syringe, adapter or gasket) was replaced.

### Parts required

#### Description

Unused DNA or RNA Chip

- **1** Make sure the syringe is tightly connected to the chip priming station.
- 2 Pull the plunger of the syringe to the 1.0 mL position (plunger pulled back).
- **3** Place an unused chip in the chip priming station.
- **4** Close the chip priming station. The lock of the latch will audibly click when it closes.
- **5** Press the plunger down until it is locked by the clip. This is shown in Figure 8 on page 134.

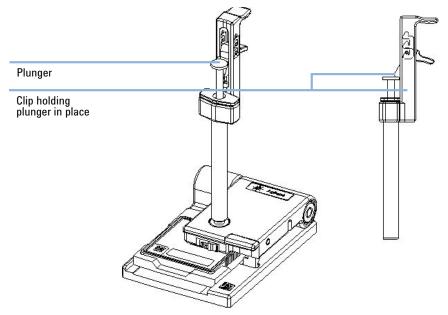


Figure 8 Locking the plunger of the syringe with the clip

Checking the Chip Priming Station for Proper Performance - Seal Test

**6** Wait for 5 seconds and lower latch of the clip to release the plunger as shown in Figure 9 on page 135.

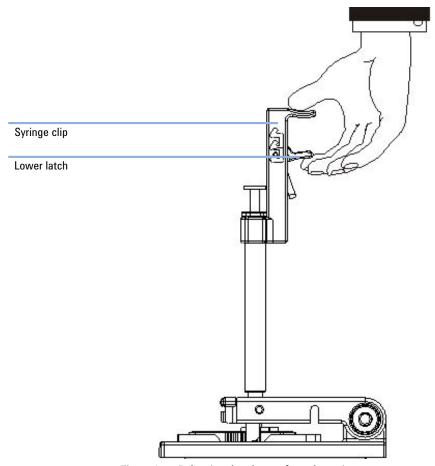


Figure 9 Releasing the plunger from the syringe

7 To indicate an appropriate sealing, the plunger should move back up at least to the 0.3 mL mark within less than 1 second.

NOTE

If the plunger does not move up to the 0.3 mL mark within a second, the syringe-chip connection is probably not tight enough. Retighten the syringe or replace the syringe adapter, syringe or gasket to fix the problem.

# 10 Maintenance of the Agilent 2100 Bioanalyzer instrument

Overview 137
Cleaning the Lens 138
Changing the Fuses 139

Overview

## Overview

## WARNING

Pathogenic, toxic, or radioactive samples

Handling and use of pathogenic, toxic, or radioactive samples and of genetically modified organisms holds risks for health and environment.

- Ensure that all necessary safety regulations, guidelines, precautions and practices are adhered to accordingly.
- Consult the laboratory safety officer for advise on the level of containment required for the application, and proper decontamination or sterilization procedures to follow if fluids escape from containers.

The Agilent 2100 Bioanalyzer instrument is classified as a *class 1 laser* product (IEC/EN 60825-1).

## WARNING

### Harmful laser light

The laser light source emits light at a power that may be harmful to the eyes.

- Avoid exposure to the beam.
- Never look into the beam or direct it towards someone else.
- System interlocks should never be disabled.
- ✓ The laser source may not be accessed.
- ✓ In case of defective instrument or laser contact Agilent Technologies.

Please refer to the *Installation and Safety Guide* for the Agilent 2100 Bioanalyzer system for more information.

The 2100 Bioanalyzer instrument should be kept clean. Cleaning should be done with a damp lint-free cloth. Do not use an excessively damp cloth allowing liquid to drip into the 2100 Bioanalyzer instrument. The following table gives an overview on the different 2100 Bioanalyzer instrument maintenance procedures:

Procedure	Time Interval	Or if
"Cleaning the Lens" on page 138	latest every 3 months	lens is contaminated with liquid spill or noticeably dirty
"Changing the Fuses" on page 139	n/a	status indicator is off and the cooling fan is not running

### Maintenance of the Agilent 2100 Bioanalyzer instrument

Cleaning the Lens

# Cleaning the Lens

Liquid spill may reduce the light throughput of the focusing lens underneath the chip. To avoid low intensity signals due to absorbent coatings on the lens, follow the procedure below.

#### When

Quarterly or after liquid has been spilled on the lens.

### Parts required

#	p/n	Description
1	NA	Reagent-grade isopropanol
1	NA	Lens tissue

- 1 Switch off the instrument. The line switch is located at the rear of the 2100 Bioanalyzer instrument.
- 2 Open the lid of the instrument.
- **3** Dampen a lens tissue with isopropanol and gently swab the surface of the lens. Repeat several times with clean tissues and alcohol each time.

### CAUTION

### Damaging the instrument

Liquid dripping into the instrument could cause a shock or damage the instrument.

- ✓ Do not allow liquid to drip into the 2100 Bioanalyzer instrument.
- 4 Wait for alcohol to evaporate before use.

### 10 Maintenance of the Agilent 2100 Bioanalyzer instrument

Changing the Fuses

# Changing the Fuses

**When** If the status indicator is off and the cooling fan is not running.

Tools required p/n Description

NA Screw driver

Parts required # p/n Description

2 2110-0007 fuses 1A, 250 V

**CAUTION** 

Disconnect the 2100 Bioanalyzer instrument from line power before changing a fuse.

✓ Use Agilent recommended fuses only.

- 1 Switch off the instrument. The line switch is located at the rear of the 2100 Bioanalyzer instrument.
- 2 Disconnect the power cable from the power input socket.

**3** To access the fuse drawer, gently lift the outer plastic housing of the power inlet socket using a screw driver, see Figure 10 on page 140.

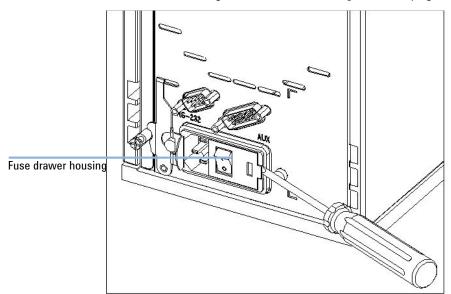


Figure 10 Remove power inlet housing

4 Pull out the fuse drawer as shown in Figure 11 on page 141.

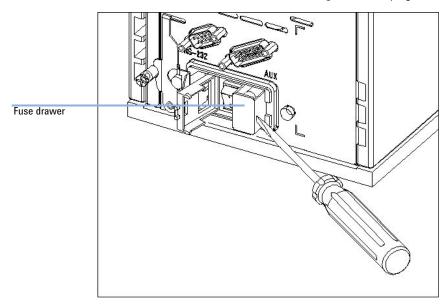


Figure 11 Remove fuse drawer

**5** Replace the two fuses.



Perform this procedure with care.

**6** Slide in the fuse drawer and push till it fits tightly as shown in Figure 12 on page 142.

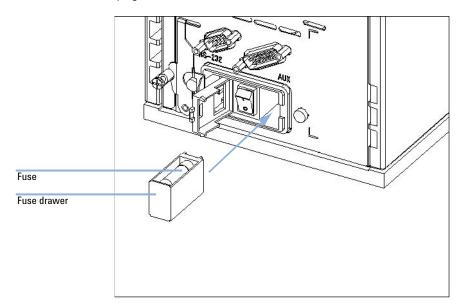


Figure 12 Insert fuse drawer

7 Close the fuse drawer housing (see Figure 13 on page 143), reconnect the instrument to the power line and switch it on.

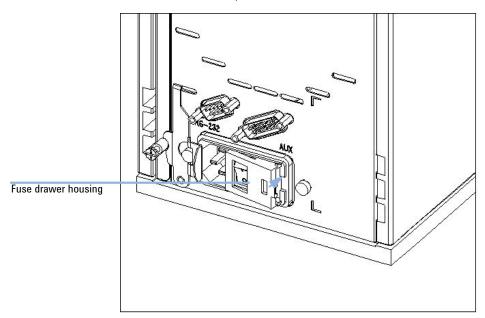


Figure 13 Close fuse drawer housing

# 11 Maintenance of the Vortexer

Changing the Adapter 145

### 11 Maintenance of the Vortexer

Changing the Adapter

# Changing the Adapter

When Whenever the vortex adapter is damaged.

Tools required	p/n		Description	
	N/A		Screw driver	
Parts required	#	p/n	Description	

1 IKA 3428300 IKA vortex mixer adapter (for MS 3 vortexers) NOTE: This part must be

purchased directly from IKA.

1 Release the 4 screws on top of the adapter.

- 2 Hold the base of the vortex mixer and pull up the head. Discard the old head according to good laboratory practices.
- 3 Place the new head adapter on the vortex mixer.
- 4 Insert and fix the 4 screws with the screw driver.

# 12 Spare Parts and Accessories

Overview 147

Overview

# Overview

"Overview" on page 147 provides a list of spare parts and accessories that are available for the 2100 Bioanalyzer system.

To buy parts, contact Agilent Technologies at <a href="https://www.agilent.com/en/contact-us/page">https://www.agilent.com/en/contact-us/page</a>.

Reorder number	Part	Description
5185-5990	Filters for gel matrix	Extra filters for gel matrix. Contains 25 spin filters for the electrophoresis assays.
5188-8031	USB-serial adapter cable	Connects RS232 cables to USB PC ports (for PCs without serial ports).
RS232-6101	RS232 cable	Communication cable between PC and instrument.
2110-0007	Fuse	Fuse for power supply.
5065-9951	Electrode cleaner kit	Contains 7 electrode cleaners for the maintenance of the electrode cartridge.
5065-4401	Chip priming station	Includes gasket kit and adjustable clip.
G2938-68716	Gasket kit	Contains spare parts for chip priming station: 1 adapter, 1 mounting ring and 10 gaskets.
5042-1398	Adjustable clip	For use with luer lock syringe.
5065-4413	Electrode cartridge	Removable cartridge with detachable 16-pin electrode assembly for easy cleaning. For use with electrophoresis assays. NOTE: electrode pin set is not sold separately.
G2938-68300	Test chip kit for electrophoresis	Comprises 1 autofocus and 1 electrode/diode chips.
IKA 3428300	Vortex mixer adapter	For IKA MS3 vortexer (must be ordered through IKA).

## Index

2 2100 expert software 15	demo port 19 diagnostic test 29 DNA symptoms 58, 33 troubleshooting 32	high sensitivity DNA 58 high sensitivity protein 107  I installation qualification 24 instrument context 15
A  additional peaks  DNA 39  protein 95  RNA 69  artefact peaks  high sensitivity DNA 59	electrode cartridge 111 electrode cleaner 8 electrode cleaning DNA 112 electrode cleaning protein 112 RNA 118, 114 error message	L ladder protein 102 lens 138 license 17 log book 32 lower marker protein 97
baseline DNA 48, 49, 46, 47 protein 104 RNA 74, 78 bioanalyzer icons 15 bioanalyzer 13, 27, 137 broad peaks DNA 45 RNA 75	DNA 57 Protein 106 RNA 80 essential measurement practices 6  F fuse drawer 141 fuse 139  G	M marker assignment DNA 54 protein 92 migration DNA 50 protein 105 RNA 79
C chip not detected DNA 38 protein 89 RNA 67	gasket 9, 131, 132 gel-dye 10 gel 10	missing peaks DNA 43 RNA 76 mounting ring 129
chip priming station 9, 127, 134 chips 12 COM port 17, 19 contamination 8  D  degraded RNA 71	handling chips 12 gel-dye 10 gel 10 reagents 10 samples 11 hardware diagnostics 27 head adapter 145	next generation sequencing 58  O optical signal protein 108

## Index

P	run log 32	T
peak tailing DNA 52 peaks broad protein 103 peaks missing protein 98 peaks	run time DNA 53 protein 91  S salt concentration 11 samples 11	test chips 27 tools and handling 8 troubleshooting DNA 32 protein 84 RNA 63
high sensitivity DNA 59 pin set cleaning 121 pipette tips 8 plunger 134 poor chip performance 37, 68 power inlet socket 140 power switch 15 proteases 86 protein symptoms 85, 107	saturation protein 108 RNA 70 seal test 134 sensitivity DNA 41 high sensitivity protein 109 protein 101 RNA 73 short circuit diagnostic test 125 signal intensity	U upper marker DNA 44 protein 96  V validation 24 verification 24 vortexer adapter 145
troubleshooting 84  Q quantitation    DNA 35, 34    protein 87, 86    RNA 66, 65  R reagents 10 reproducibility    protein 100 RNA fragment 77 RNA pico 70 RNA    symptoms 64    troubleshooting 63 RNAse contamination 117, 120 RS232 cable 17, 19 run aborted 37, 68, 90	DNA 41 high sensitivity protein 109 protein 101 RNA 73 sizing DNA 36 protein 88 small RNA 70 spikes DNA 40 protein 99 RNA 72 split peaks high sensitivity DNA 60 status indicator 15 syringe adapter 129, 131, 132 syringe 8, 128	

### In this Book

This manual provides maintenance and troubleshooting information for the Agilent 2100 Bioanalyzer system. It includes essential measurement practices, troubleshooting hints for hardware, software and applications, maintenance procedures and a list of spare parts and accessories.

This manual is based on the 2100 Expert Software revision B.02.08 and newer.

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