Agilent 2100 Bioanalyzer System

Maintenance and Troubleshooting Guide

Agilent Technologies

Notices

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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. Other software revisions may have an impact on results.

Contents

1 Essential Measurement Practices

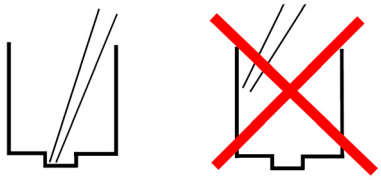
Overview

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, go to the Agilent web site at:

www.agilent.com/genomics/bioanalyzer

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.

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 131. If necessary, replace the gasket and/or adapter.

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.

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.

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 µL of sample buffer to each unused sample well so the total liquid volume in each well is at least 6 µL.

  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

- 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).
- Do not force the chip to fit in the 2100 Bioanalyzer instrument. The electrode cartridge may be damaged when the lid is closed.
- Clean electrodes on a daily basis using the electrode cleaner. For more details, see "Maintenance of the Electrode Cartridge" on page 115.
- Thorougly 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 126.
- Clean the focusing lens once a month (or after any liquid spill) using isopropanol. For more details, see "Cleaning the Lens" on page 143.

2
Troubleshooting the Instrument Communication

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 instument 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.

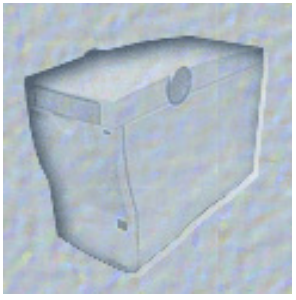
3 Select the instrument tab in the **Instrument** context.

4 In the tree view, highlight the appropriate instrument.

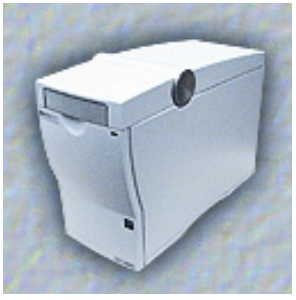
The connection to the selected instrument is established.

5 Open and close the lid – the icon in the **Instrument** context should change from closed to open, see Table 1 on page 16.

Table 1 2100 Bioanalyzer instrument icons



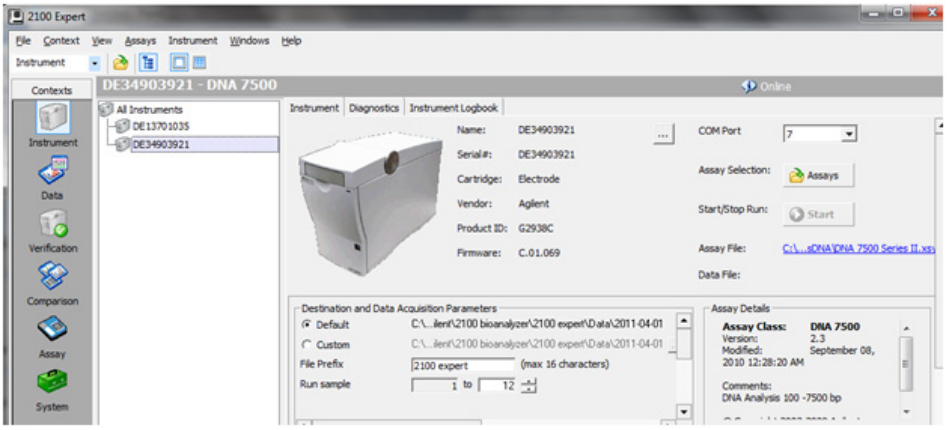
Switched off
or not connected to PC.



Online
and lid closed.

Online
and lid open.

If the instrument is connected successfully, additional hardware information (serial number, cartridge type,...) is displayed on top of the screen, see Figure on page 17.



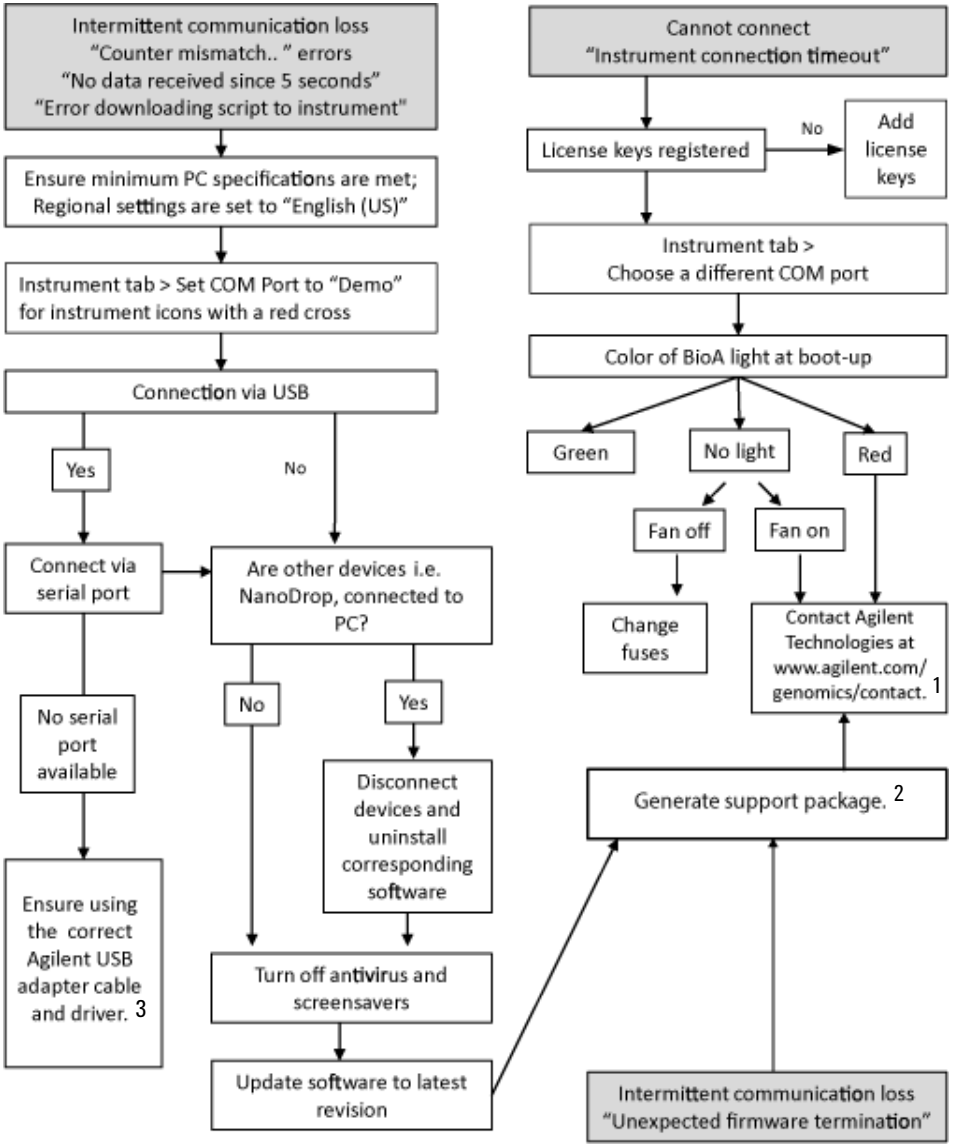
If the icon does not change, the instrument is not connected successfully. For solutions, see “Overview” on page 18.

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 20.
- 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 www.agilent.com/genomics/contact.
- 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 144. A set of spare fuses comes with the instrument. If the status indicator is off and the fan is running, contact Agilent Technologies at www.agilent.com/genomics/contact.
- 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 www.agilent.com/genomics/contact.

Troubleshooting Communication Issue Flow Chart



1	www.agilent.com/genomics/contact
2	see "How to create a Support Package" on page 22
3	see "USB to Serial Adapter" on page 21

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 17
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 22) and contact Agilent Technologies at www.agilent.com/genomics/contact.

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

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 greater

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 greater.

If the PC is connected to the internet, the correct driver will install automatically when the USB-Serial Adapter cable is plugged into the USB port of your PC.

For off-line systems, please install the driver from the Agilent 2100 Expert Software CD by clicking on the executable file found in SupportDriver/88-8031CDM20824.exe. If a CD is not available, download the driver.

Install the driver (http://www.genomics.agilent.com/article.jsp?pageId=2353) prior to connecting the adapter and the 2100 Bioanalyzer instrument and proceed as described below.

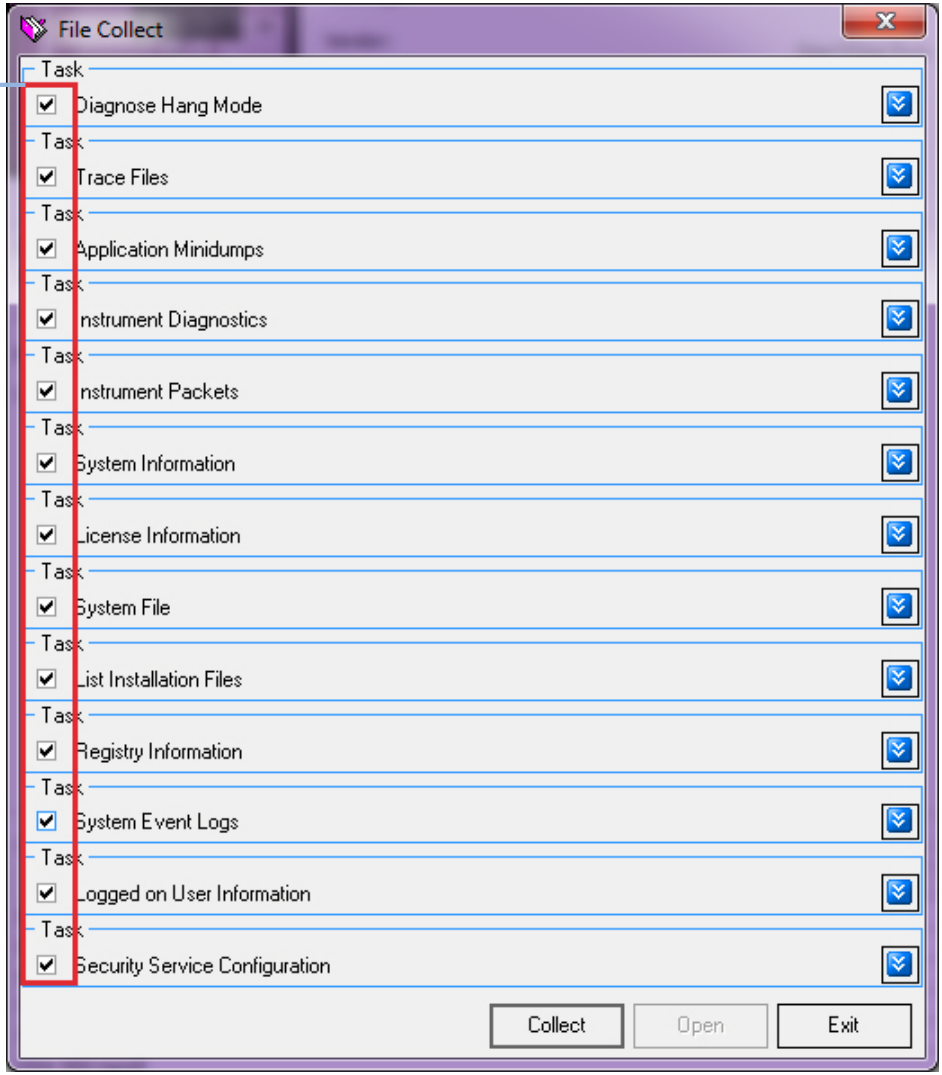
- Close the 2100 Expert Software.
- Execute the installation program of the driver and follow the instructions. Reboot the operating system.
- Physically connect the 2100 Bioanalyzer instrument and the laptop by using the USB-Serial Adapter cable and the standard Serial RS-232 cable.

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.

Make sure all tasks are selected



NOTE

Alternatively:

- In the Windows Start menu, open All Programs > Agilent 2100 Bioanalyzer > Utilities > 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

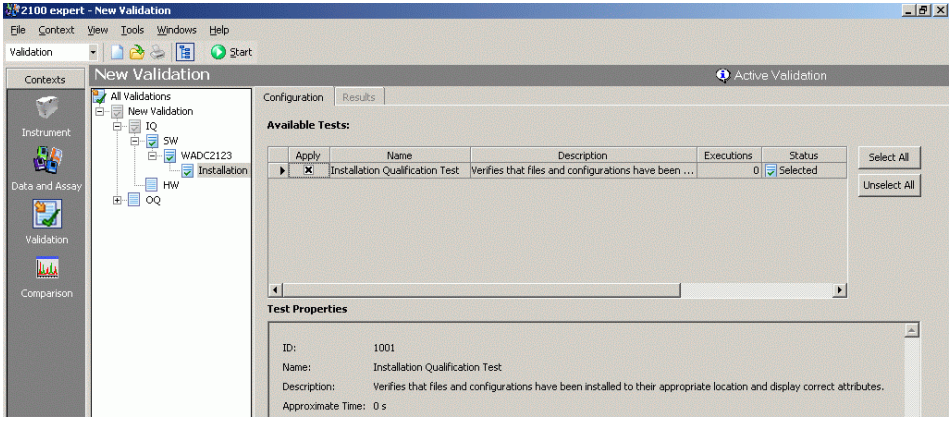
Run Installation Qualification Test

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**.

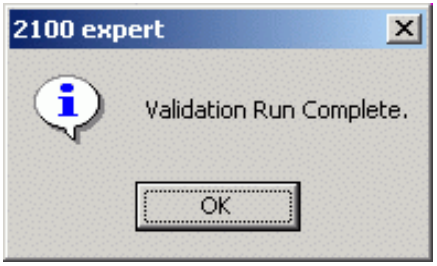
3 Under **Available Tests** select the checkbox of the **Installation Qualification Test**. This test verifies that files and configurations have been installed to their appropriate locations and display correct attributes.



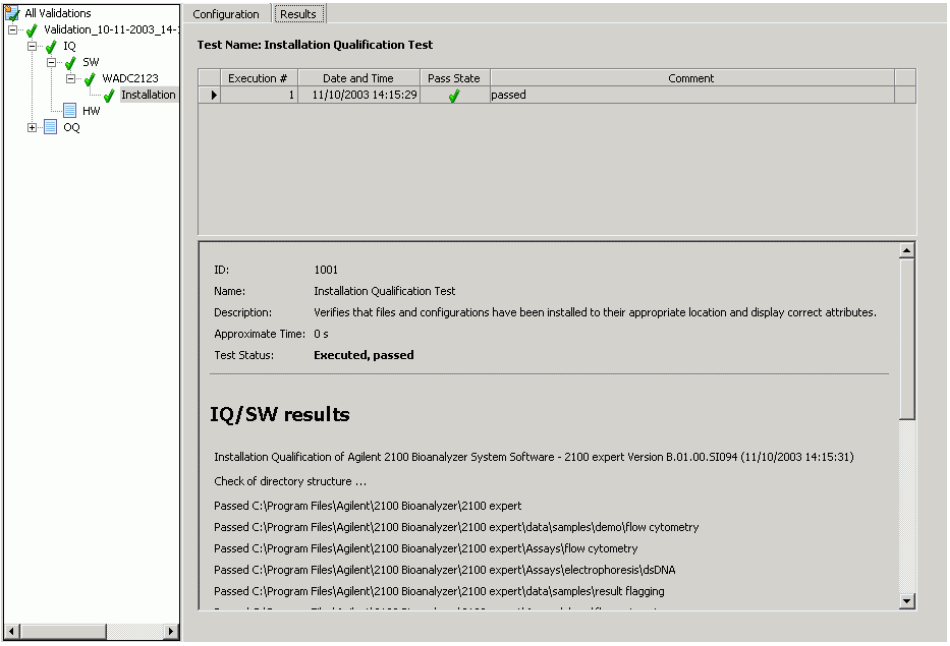
4 Start the software test tool by clicking **Start**.

5 The **Save As** dialog box appears. Define the name and location of the verification file.

6 When the test is finished, the **Validation Run Complete** message appears.



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 16 and "Overview" on page 30 for further troubleshooting procedures. Finally, to check the application, see "Overview" on page 36, "Overview" on page 68 , or "Overview" on page 88.

9 If the test fails, reinstall the 2100 Expert Software using the software CD-ROM that is supplied with the system. Follow the instructions that are printed on the CD-ROM.

10 If the test continues to fail, contact Agilent Technologies at www.agilent.com/genomics/contact.

4
Hardware Diagnostics

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 30 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

Table 3 on page 31 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

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.

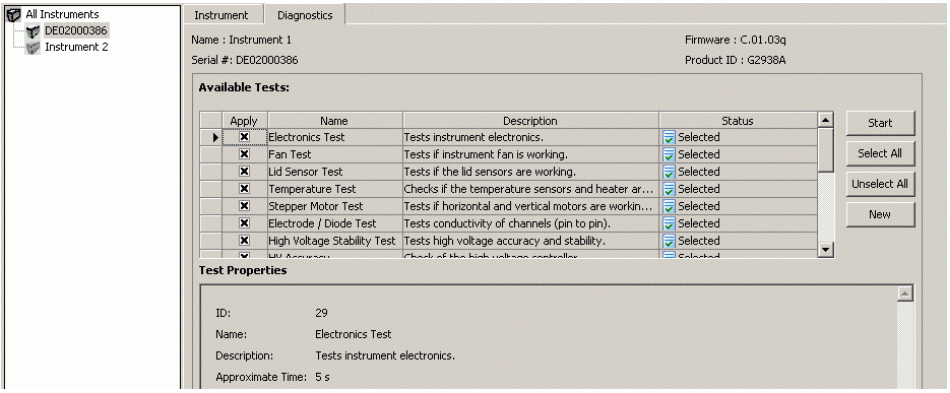
1 Start the 2100 Expert Software.

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.

7 If there are failures, repeat the failed tests.

8 If failures persist, contact Agilent Technologies at www.agilent.com/genomics/contact.

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

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 37.

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)

Click to go straight to the troubleshooting hints.

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 48.
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 37

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.

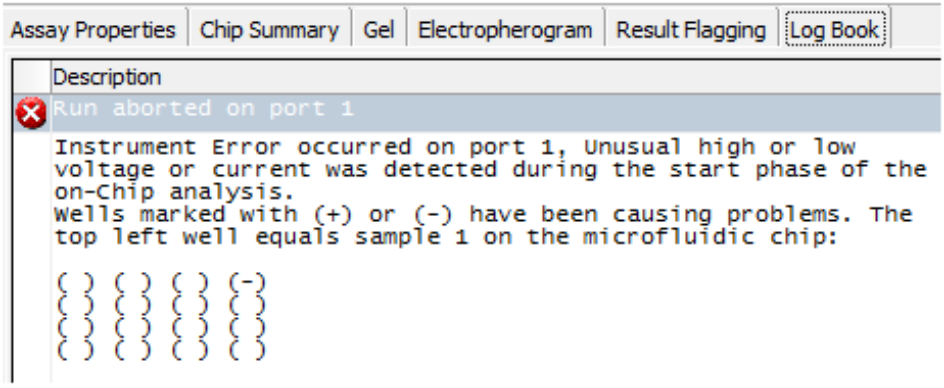
Back to “Symptoms (DNA)” on page 37

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 139. 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 58
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 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (DNA)" on page 37

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.

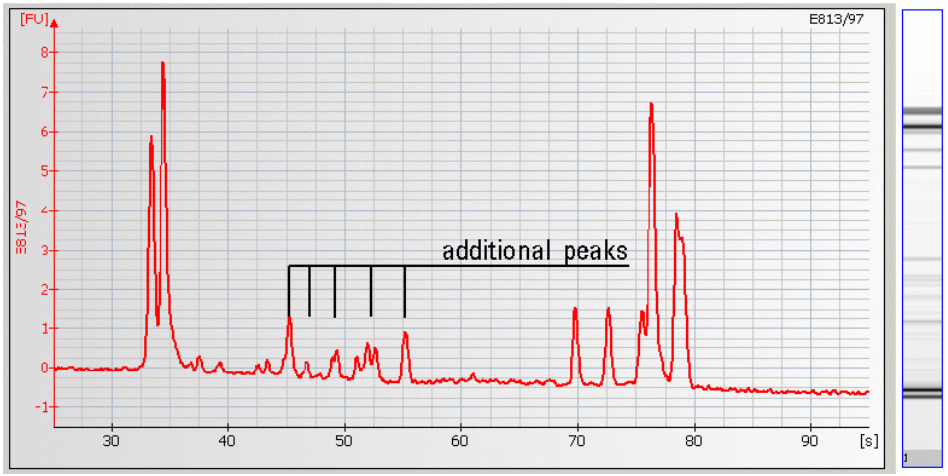
Most probable causes	*Solution*
Insufficient volume in well(s).	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain sufficient ladder, samples or buffer.
Air bubble at the bottom of the well, obstructing access to microchannels.	Always insert the pipette tip to the bottom 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 126.
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 139. 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 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Chip Not Detected

Most probable causes	*Solution*
No communication between instrument and PC.	Check instrument communication as described in "Verify the Instrument Communication" on page 16.
Insufficient volume in well(s).	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain ladder, samples or buffer.
Chip not properly primed. Air bubble in chip.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 139. 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*
Expired reagents.	Prepare new chip with fresh reagents.
Least probable causes	*Solution*
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (DNA)" on page 37

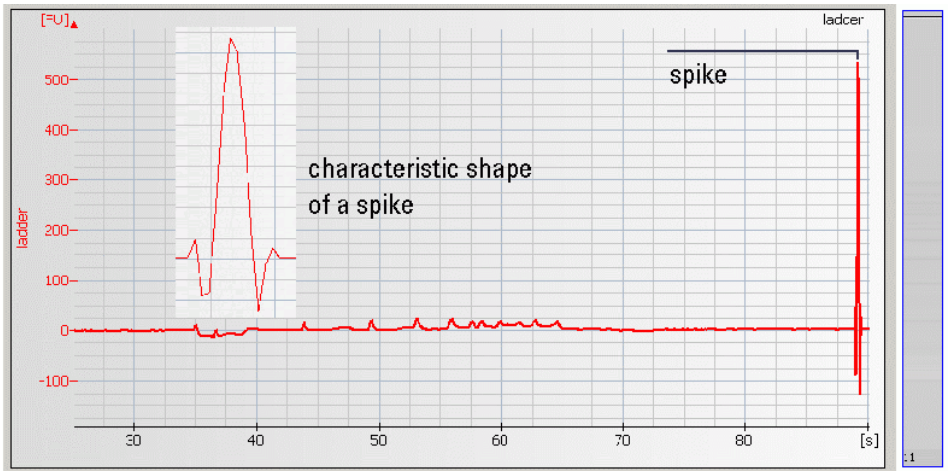
Additional Sample or Ladder Peaks



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 139. 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.

Back to "Symptoms (DNA)" on page 37

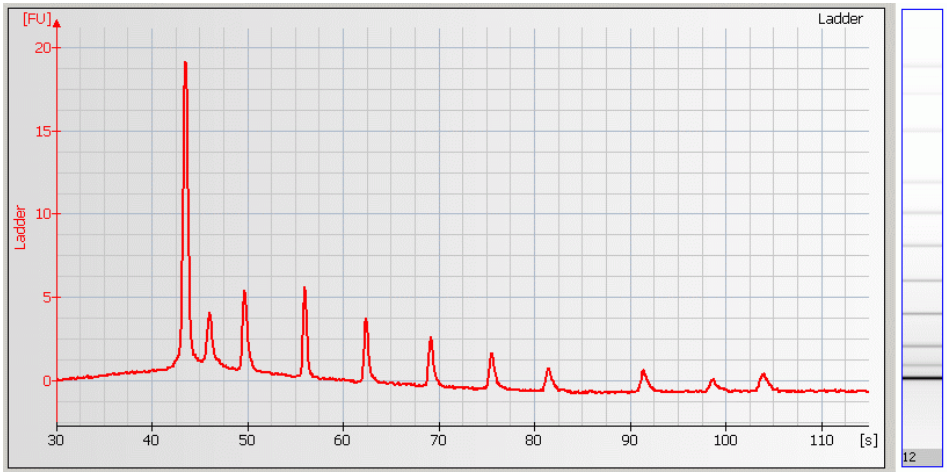
Spikes



Most 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.
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 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.
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 (DNA)” on page 37

Low Signal Intensity

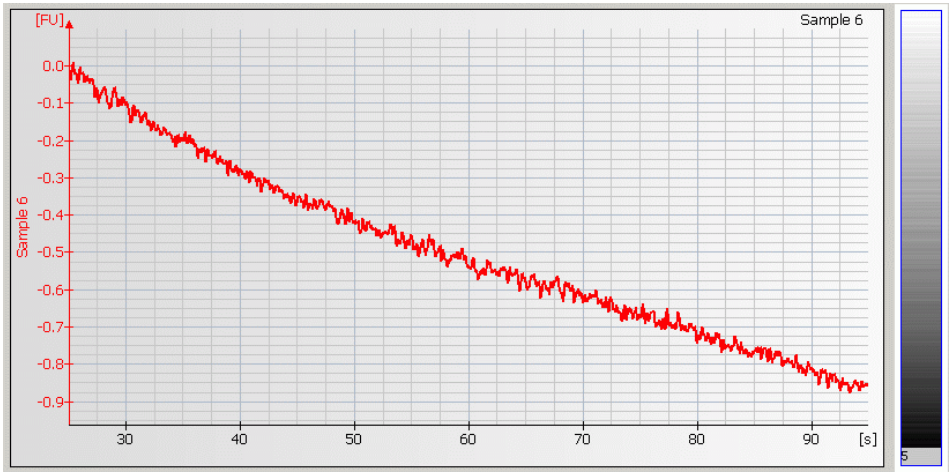


Most probable causes	*Solution*
Dye concentration too low.	Use dye concentration according to the Kit Guide. Let the dye warm up to room temperature for 30 minutes before preparing the gel-dye mix. Protect the dye from light during this time.
Pipetting error during preparation of reagent mixes.	Check 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 143. 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*
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.
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.
Autofocus failure.	Check autofocus using the "Hardware Diagnostics" on page 29. If autofocus fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (DNA)" on page 37

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 139. 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 126.
Least probable causes	*Solution*
Laser broken.	Perform Optics, Autofocus, and Laser Stability tests as described in "Hardware Diagnostics" on page 29. If tests fail, contact Agilent Technologies at www.agilent.com/genomics/contact.
Autofocus failure or high voltage power supply defective	Check autofocus and high voltage power supply by means of the "Hardware Diagnostics" on page 29. If a diagnostic test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

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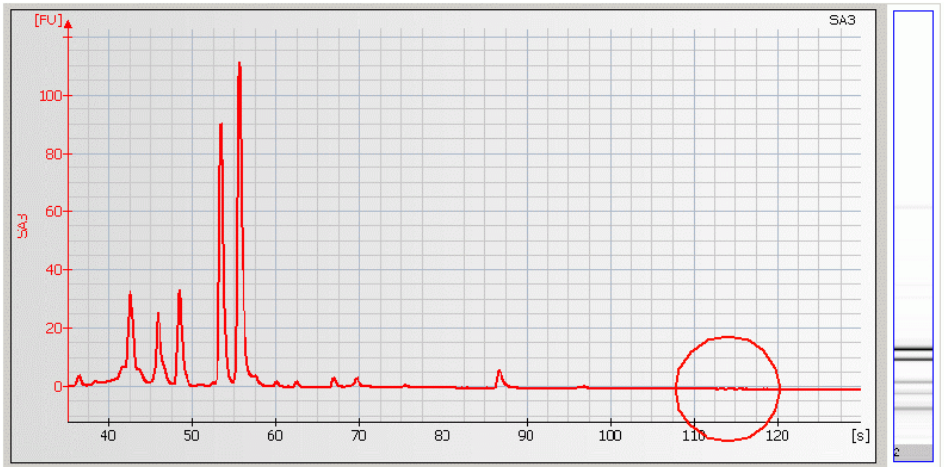
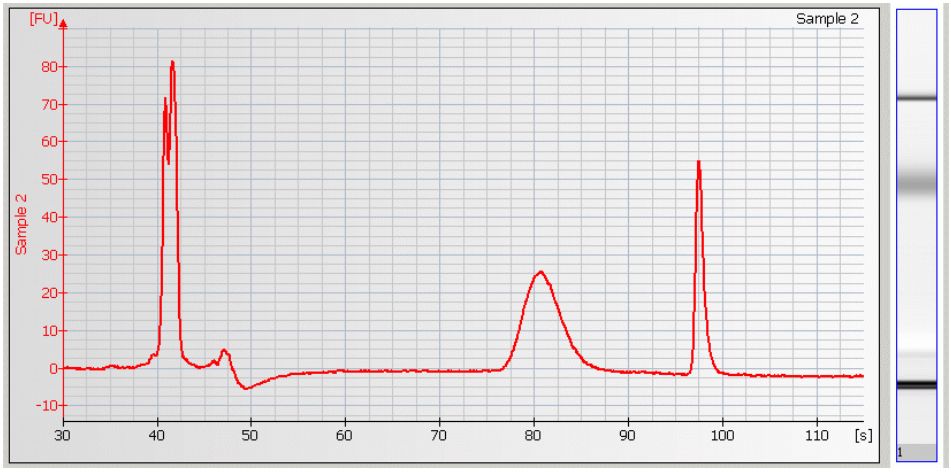
Missing Upper Marker



Most probable causes	*Solution*
Alignment of upper marker not set properly.	Manually assign upper marker. Follow instructions for "Manual Marker Assignment" on page 58
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 37

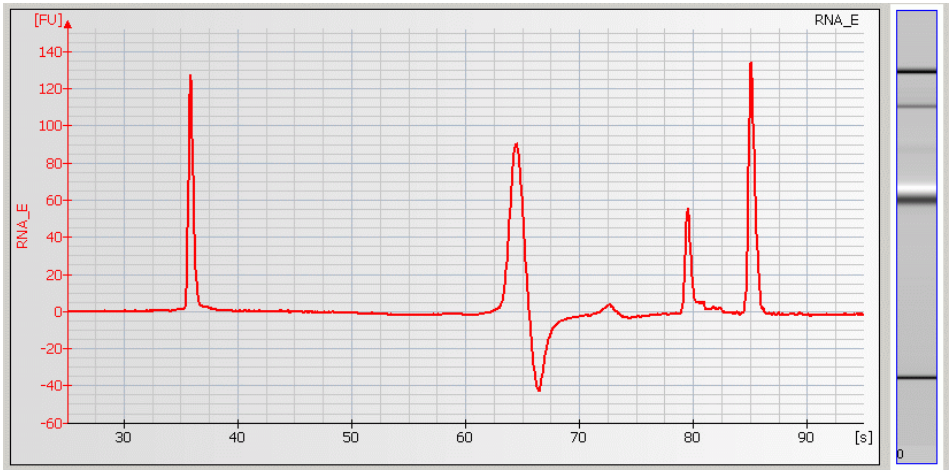
Broad Peaks



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 126.
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 139. 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 37

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 30. If the diagnostic test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

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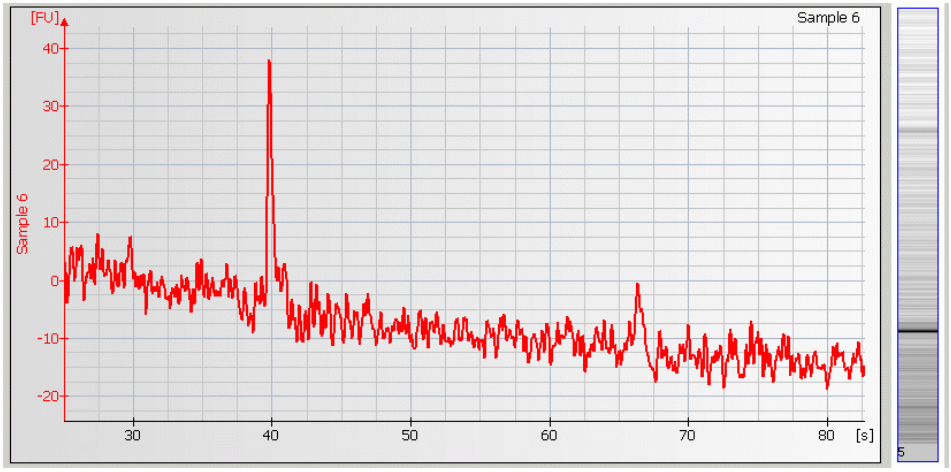
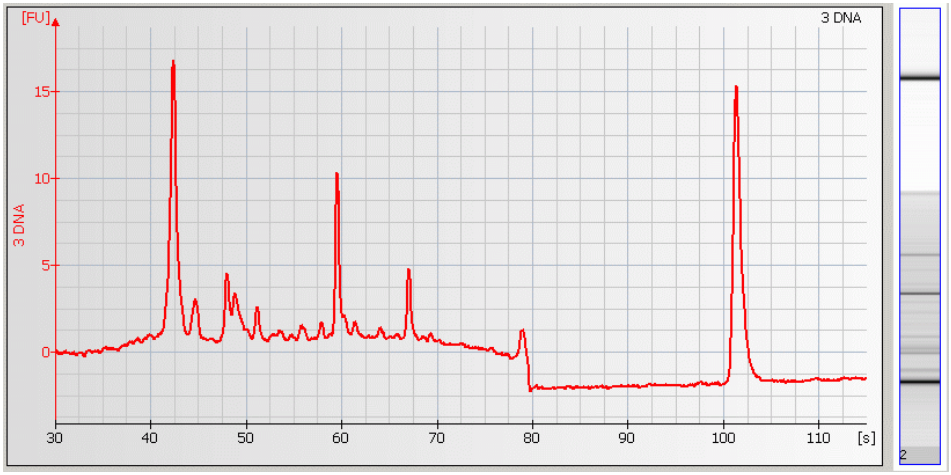
Baseline Noise



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 143. 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 29. If tests fail, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (DNA)" on page 37

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 29. If the diagnostic test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

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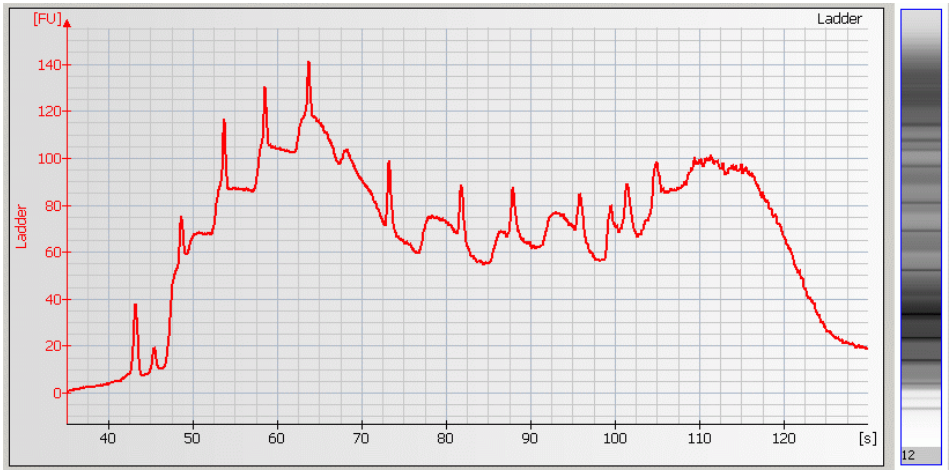
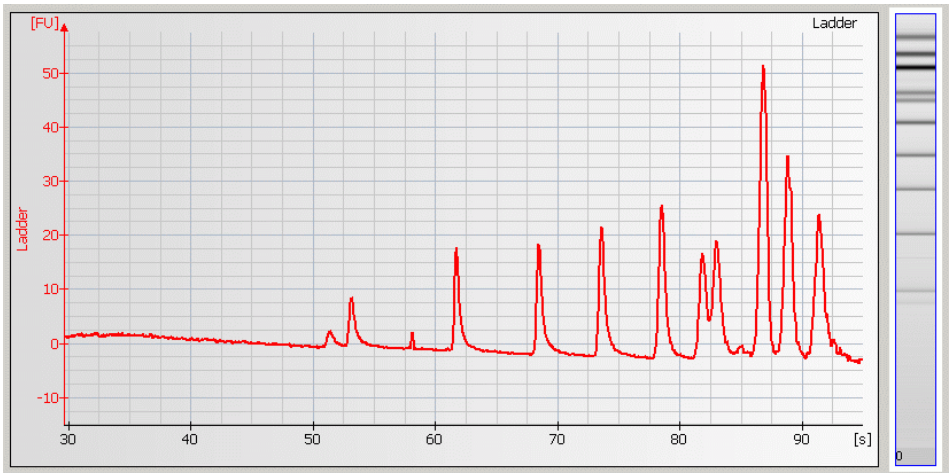
Wavy Baseline



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 126.
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 139. 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 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.
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (DNA)" on page 37

Late Migration

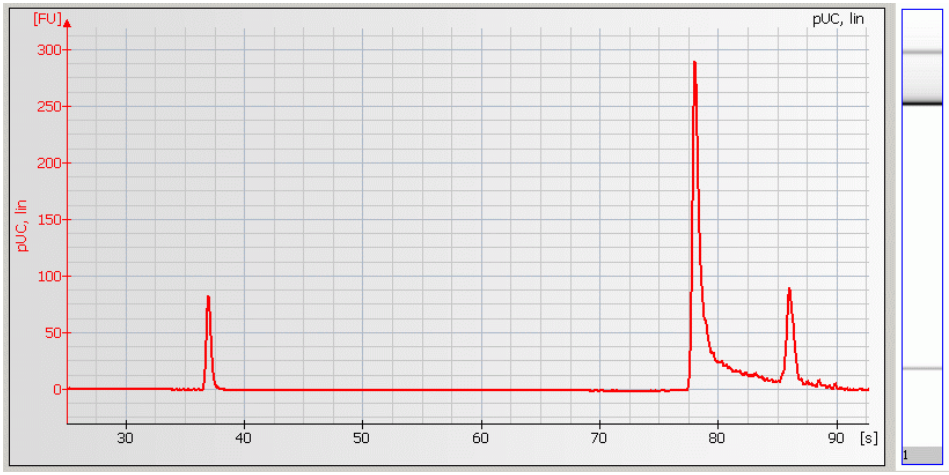


Most probable causes	*Solution*
Chip not properly primed. Clogged chip priming station or wrong priming station settings.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 139. Clean/replace syringe, gasket and 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 126.
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*
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.
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.
Vortex speed too high.	Vortex chip for 1 minute. Only use the IKA vortexer. Ensure speed is adjusted to the setpoint.

Genomic DNA or high molecular weight DNA contamination.	Check DNA isolation protocol.
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 149.
Changes in ambient temperature of more than 5°C during the run.	Place the 2100 Bioanalyzer instrument in a thermally stable environment.

Back to "Symptoms (DNA)" on page 37

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.

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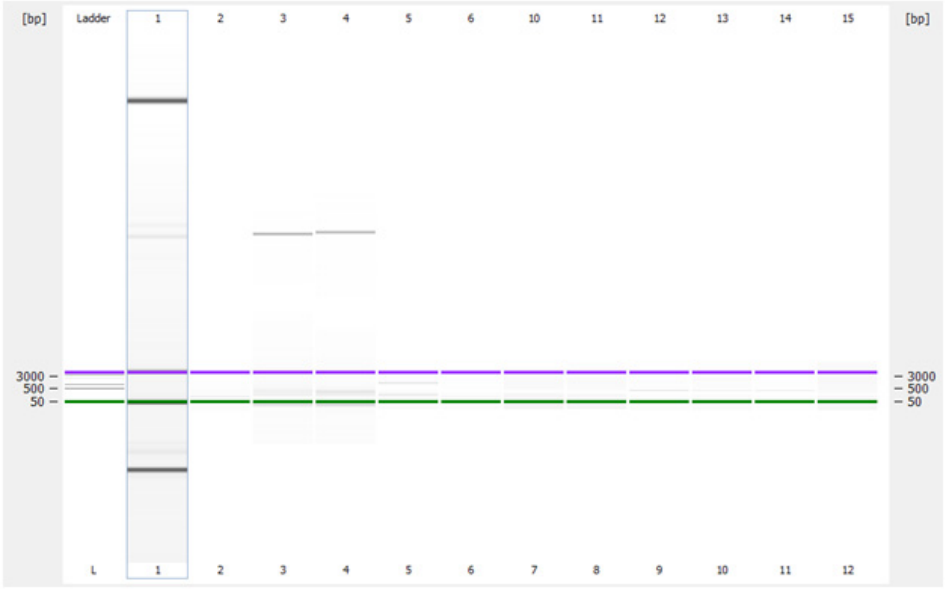
Unexpected Run Time

Unexpected Run Time

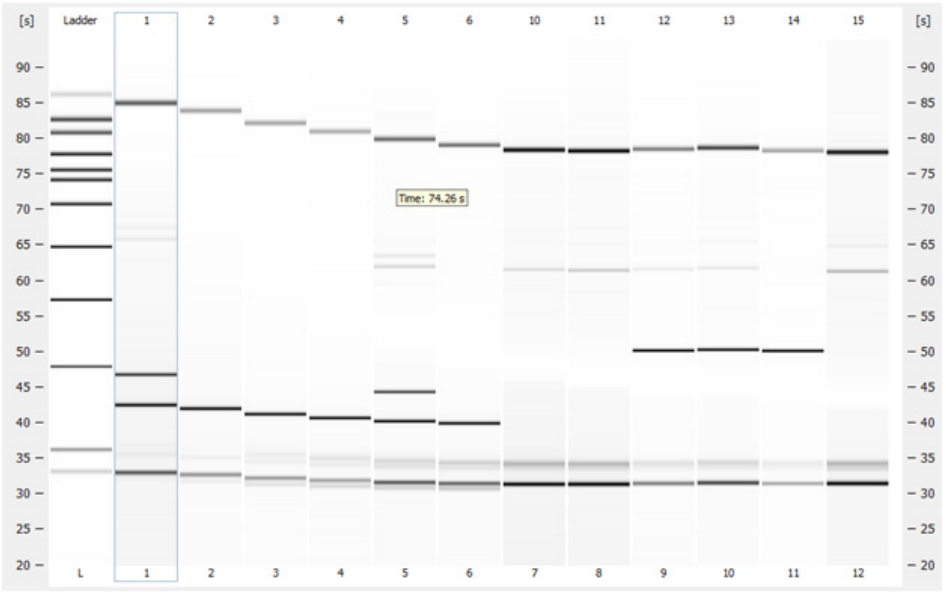


Most probable causes	*Solution*
Lower and/or upper markers are called incorrectly.	Turn off alignment and check which bands are the correct lower and upper markers. For more details, see "Manual Marker Assignment" on page 58.

Back to "Symptoms (DNA)" on page 37

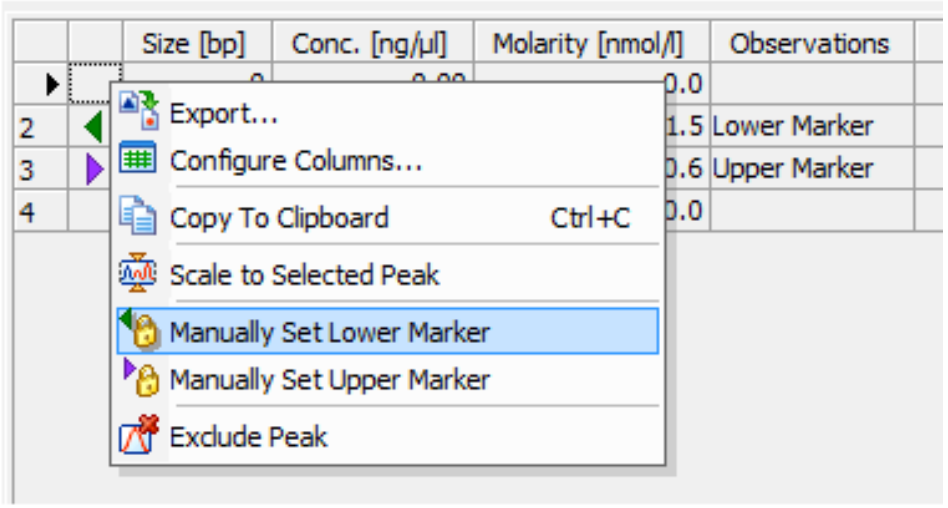
Manual Marker Assignment

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

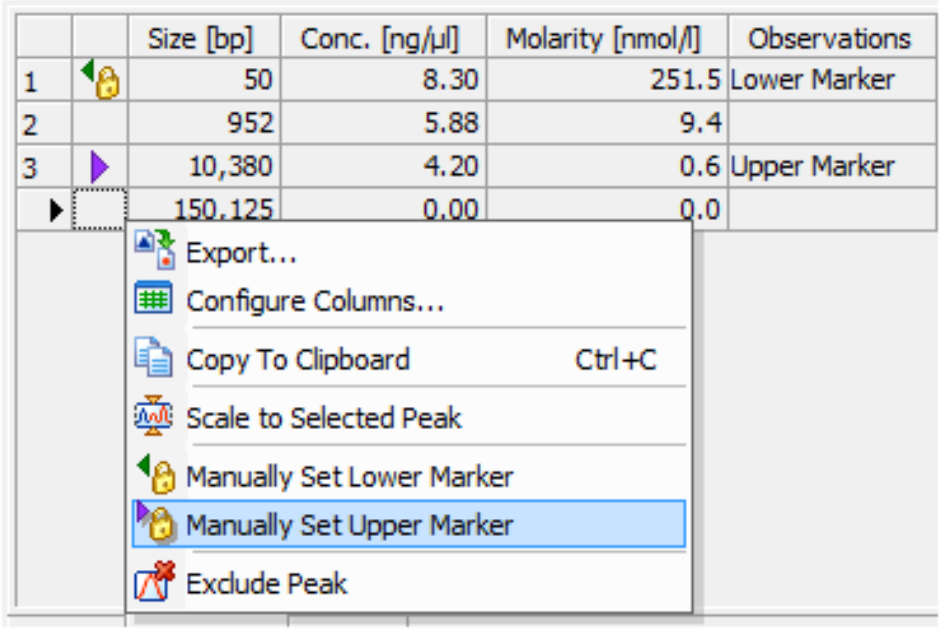


2 Turn the alignment back on  . Check the electropherogram of each sample. Go to the **Peak Table** tab to adjust the markers.

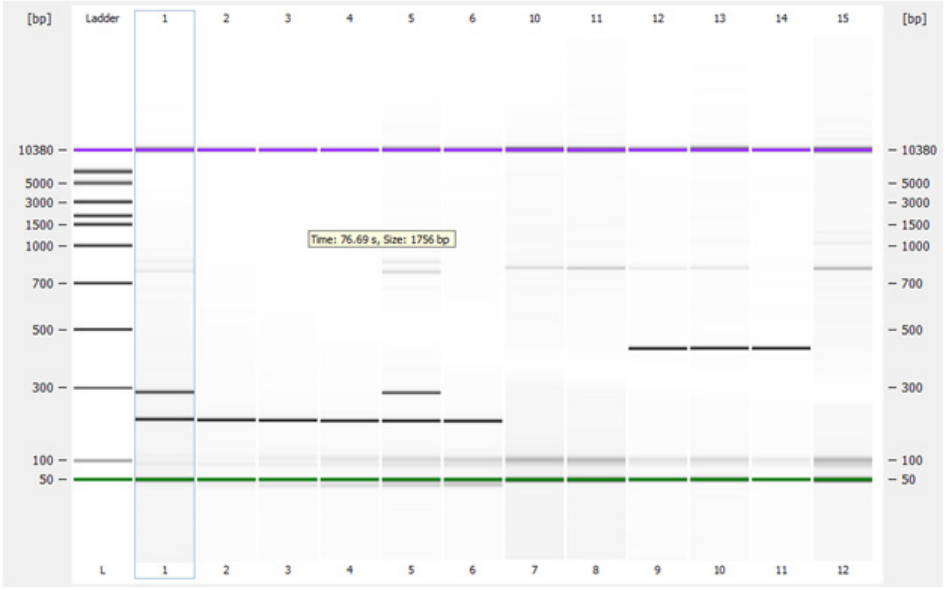
3 To adjust the lower marker, right click on the correct peak, and choose **Manually Set Lower Marker**.



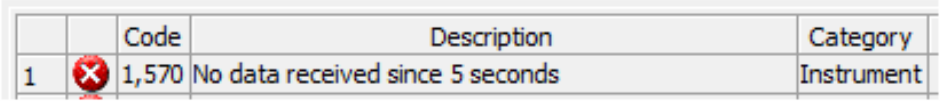
4 To adjust the upper marker, right click on the correct peak, and choose **Manually Set Upper Marker**.



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



Error Message: No data received since 5 seconds



		Code	Description	Category
1		1,570	No data received since 5 seconds	Instrument

Most probable causes	*Solution*
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 15 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 21.

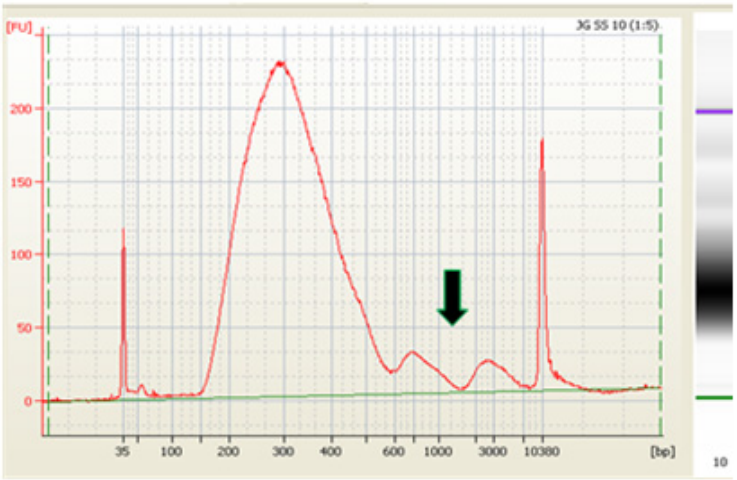
Symptoms (High Sensitivity DNA)

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 63
-  "Split Peaks" on page 64
-  "Baseline Negative Dips" on page 65

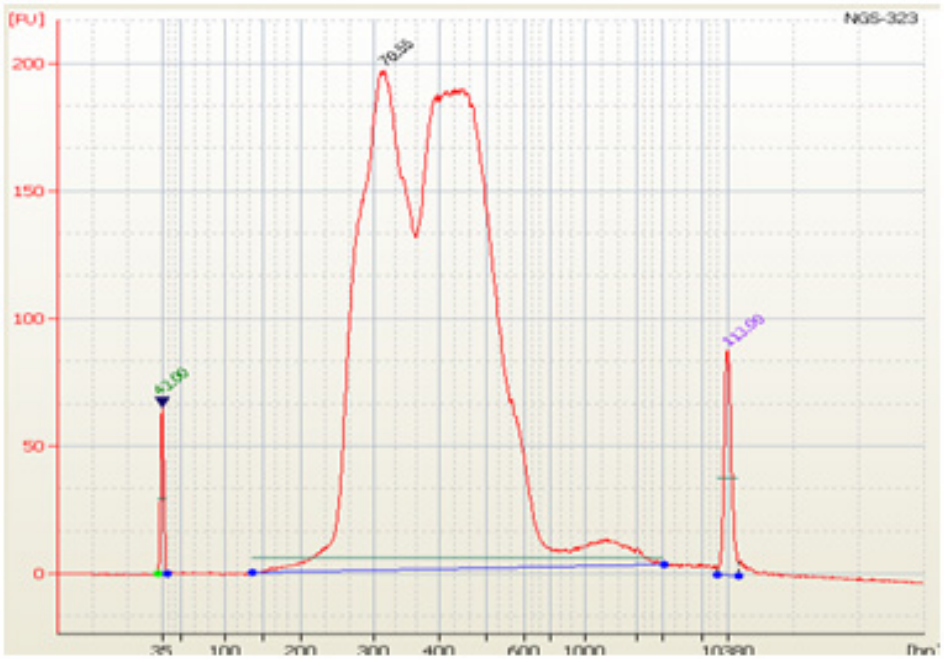
Artefact Peaks



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 62

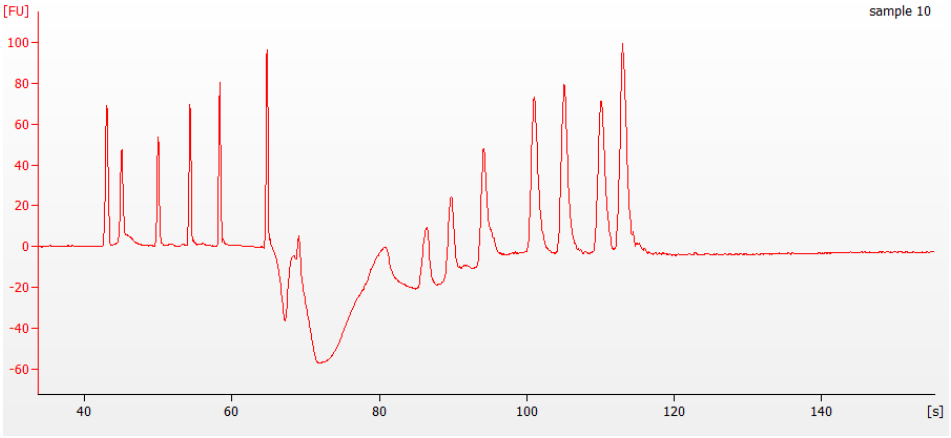
Split Peaks



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 62

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 126. 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

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 69.

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 (RNA)

Click to go straight to the troubleshooting hints.

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 126.
Electrodes contaminated with RNases.	Clean electrodes with RNaseZAP. Follow decontamination procedure, see "How to Clean the Pin Set of the Electrode Cartridge" on page 126
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.

Back to "Symptoms (RNA)" on page 69

Too Low Quantitation Results

Most probable causes	*Solution*
Reference measurement (e.g. UV absorption) was elevated due to contaminants in sample.	Purify sample prior to measurement.
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 dispensing the liquid.
Insufficient vortexing of chip.	Vortex chip for 1 minute. Only use the IKA vortexer. Adjust the speed to 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*
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.

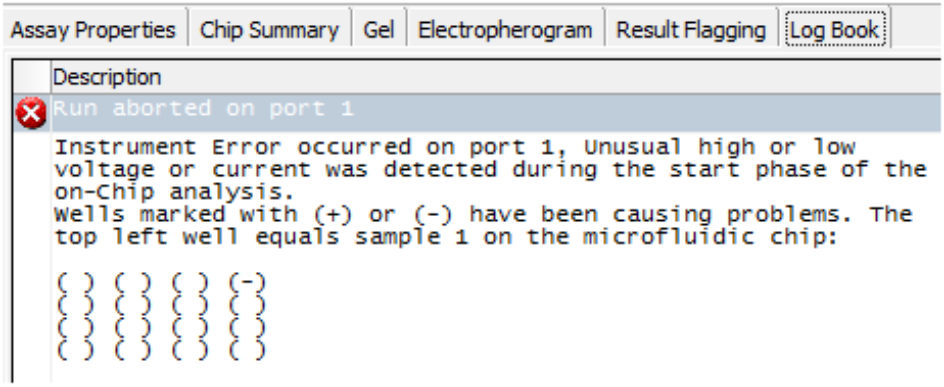
Back to “Symptoms (RNA)” on page 69.

Chip Not Detected

Most probable causes	*Solution*
Insufficient volume in well(s) or chip is empty.	Check Kit Guide for the amount of liquid to be pipetted. Ensure all wells contain ladder, samples or buffer.
Chip not properly primed. Air bubble in chip.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 139. 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*
No communication between the instrument and PC.	Test the PC-instrument communication as described in "Verify the Instrument Communication" on page 16.
Least probable causes	*Solution*
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (RNA)" on page 69

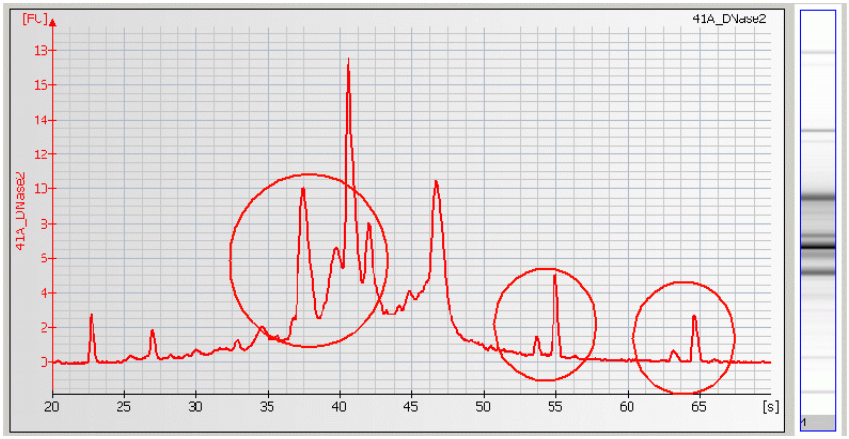
Run Aborted



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 126.
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 139. 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 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

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 126.

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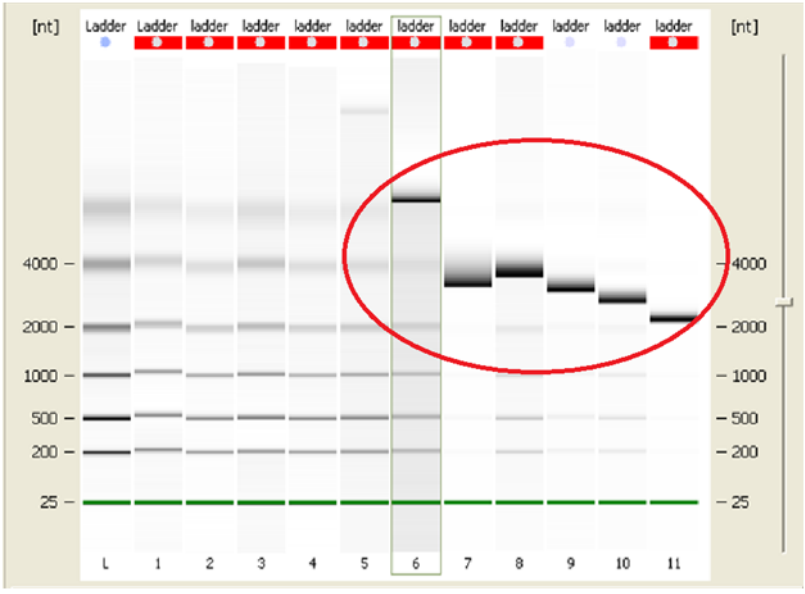
Additional Saturating Bands

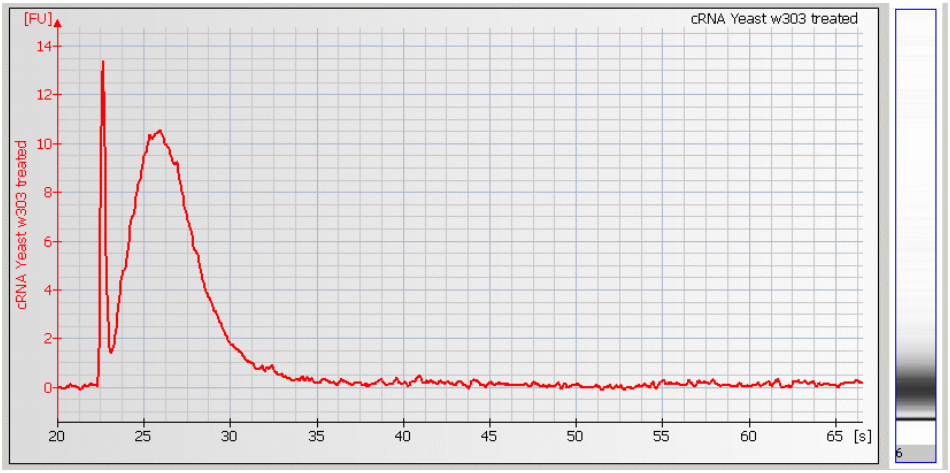
NOTE Only present in RNA 6000 Pico or Small RNA assays.



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 126.

Back to "Symptoms (RNA)" on page 69

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 126. 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.

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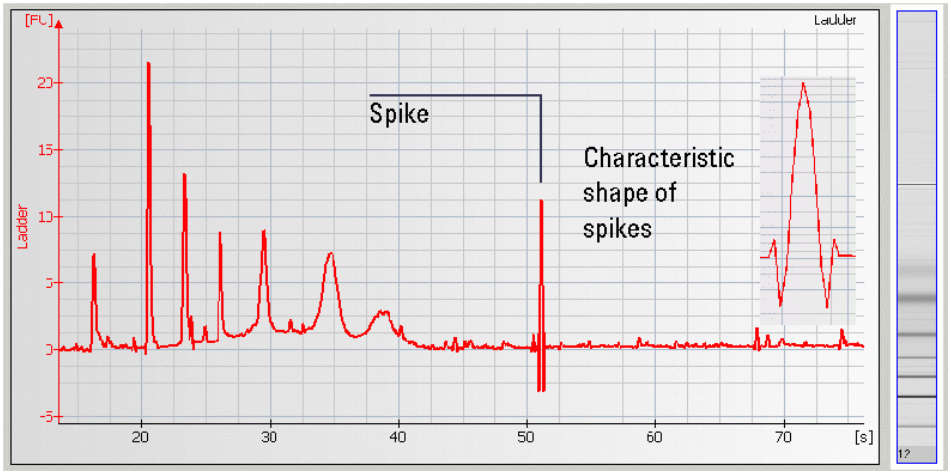
Spikes



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.

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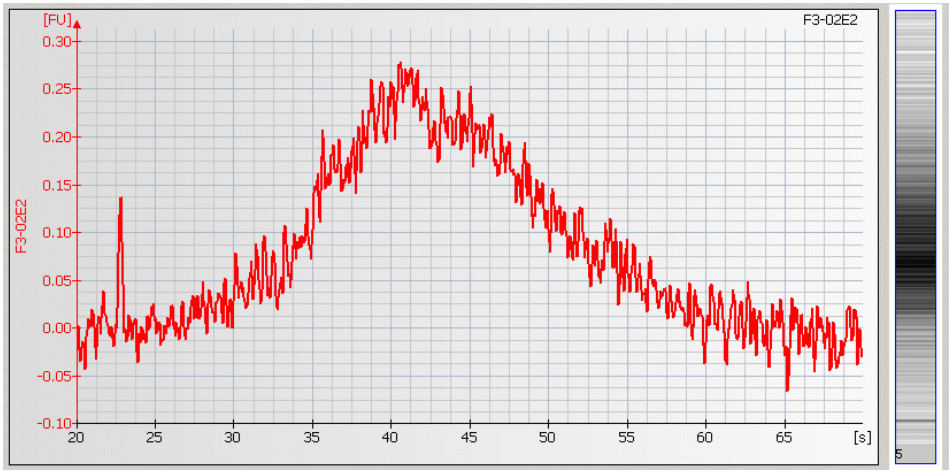
Low Signal Intensity



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 143. 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 30. If laser stability diagnostic tests fail, contact Agilent Technologies at www.agilent.com/genomics/contact.

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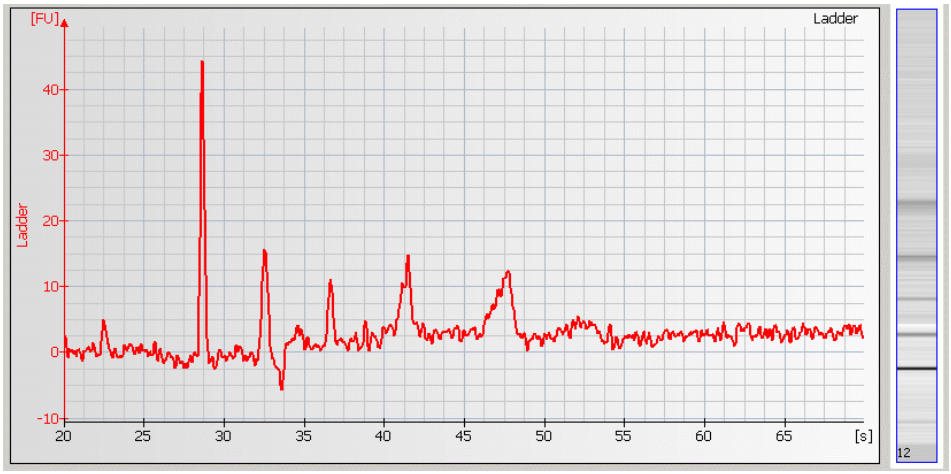
Baseline Noise



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 143. 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.

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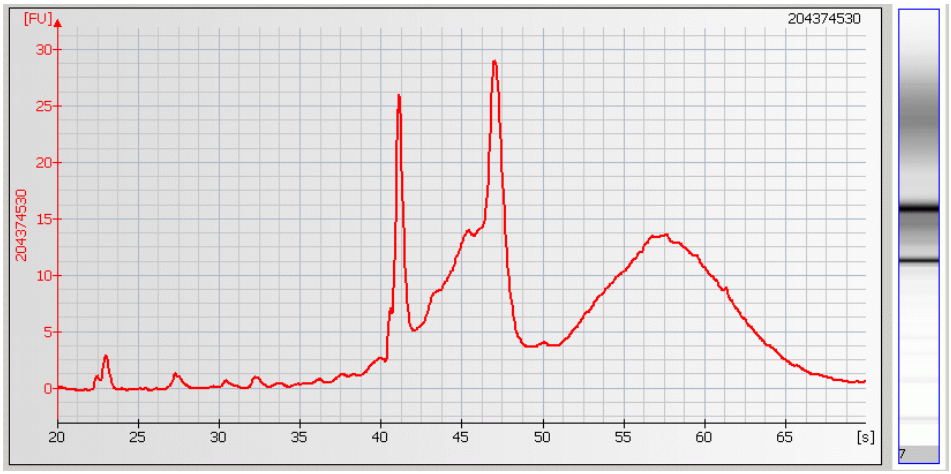
Broad Peaks



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 126
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 139. 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.

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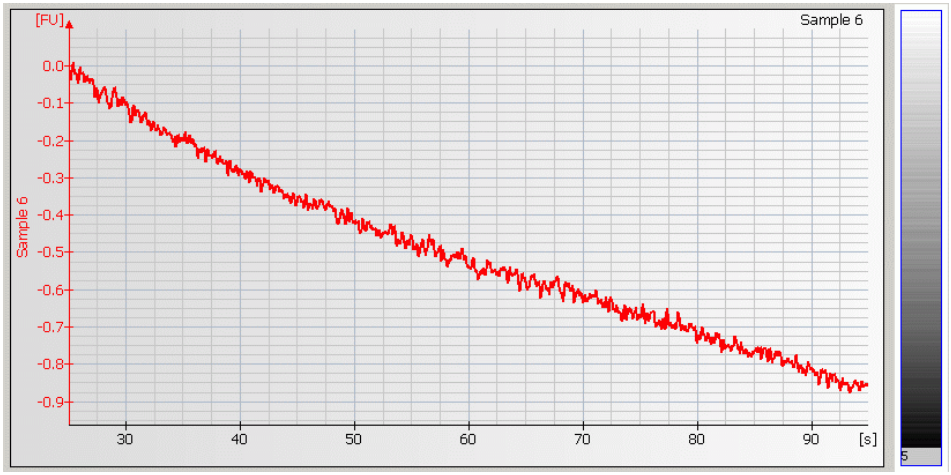
Missing Peaks



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 139. 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 126.
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 29. If tests fail, contact Agilent Technologies at www.agilent.com/genomics/contact.
Autofocus failure or high voltage power supply defective.	Check autofocus and high voltage power supply by means of the "Hardware Diagnostics" on page 29. If diagnostic fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

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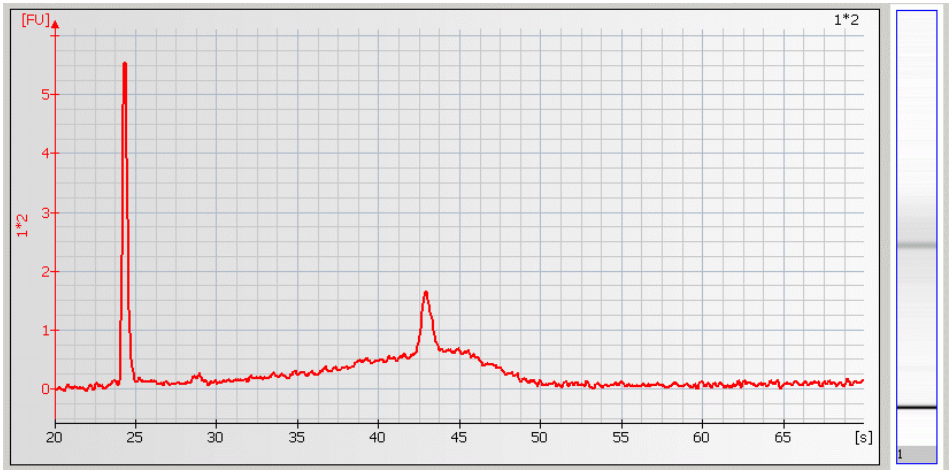
Missing RNA Fragment



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 126. Prepare a new chip with fresh reagents. Wear powder-free gloves when preparing the chip.

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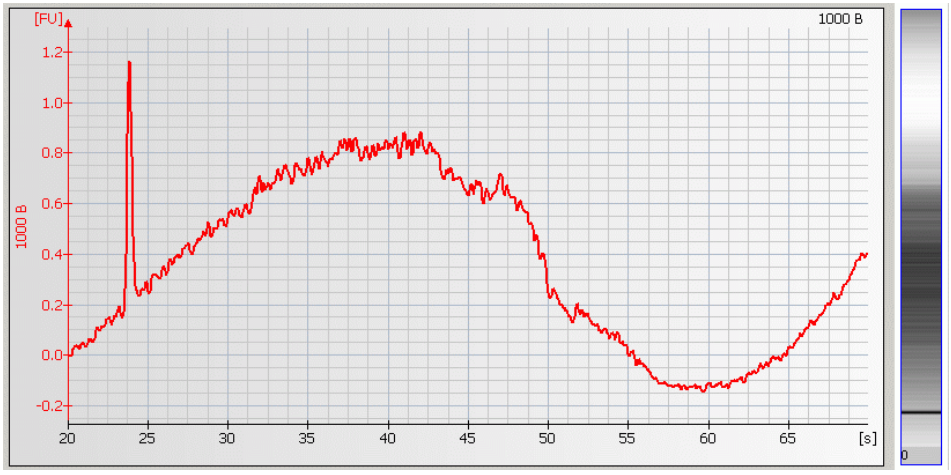
Wavy Baseline



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 126. 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 139. 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 30. If a diagnostic test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

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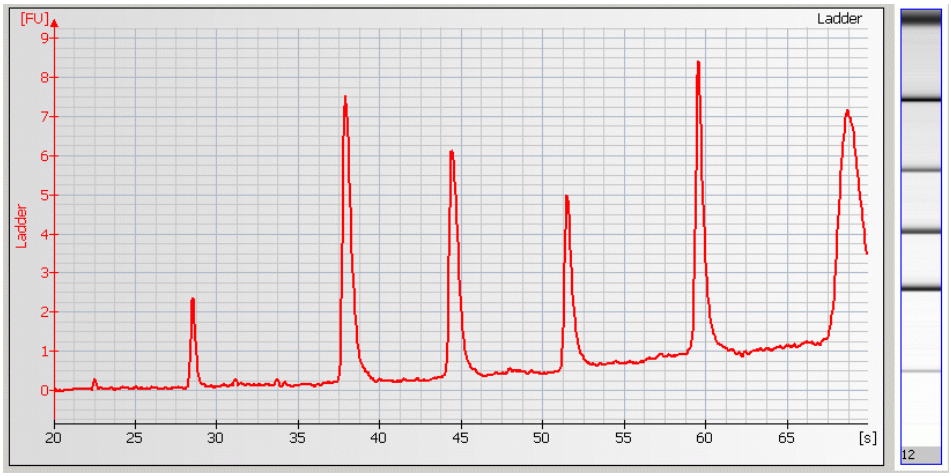
Late Migration



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 139. 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 126.
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 150.
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.

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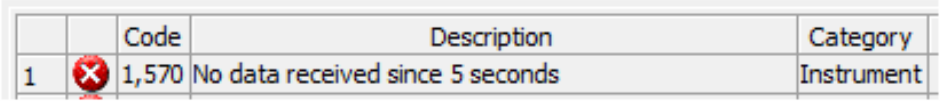
Error Message: No data received since 5 seconds



		Code	Description	Category
1		1,570	No data received since 5 seconds	Instrument

Most probable causes	*Solution*
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 15 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 21.

7
Troubleshooting the Protein Application

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 89.

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.

Symptoms (Protein)

Click to go straight to the troubleshooting hints.

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 96 .
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.

Back to "Symptoms (Protein)" on page 89

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 96.
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.

Back to "Symptoms (Protein)" on page 89

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 or 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 96.
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.

Back to "Symptoms (Protein)" on page 89

Chip Not Detected

Most probable causes	*Solution*
Insufficient volume in well(s) or chip is empty.	Check Kit Guide on amount of liquid to be pipetted. Ensure all wells contain sample, ladder or buffer.
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 well do not affect the assay).
Probable causes	*Solution*
No communication between the instrument and PC.	Test the PC-instrument communication as described in "Verify the Instrument Communication" on page 16.
Chip not properly primed. Air bubble in chip.	Prepare a new chip. Check chip priming station as described in "Checking the Chip Priming Station for Proper Performance - Seal Test" on page 139. 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*
High voltage power supply defective.	Check high voltage power supply using the "Overview" on page 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

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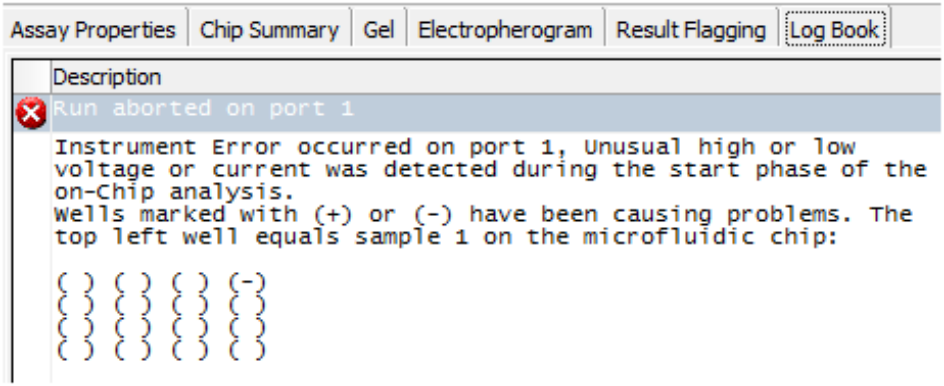
Run Aborted

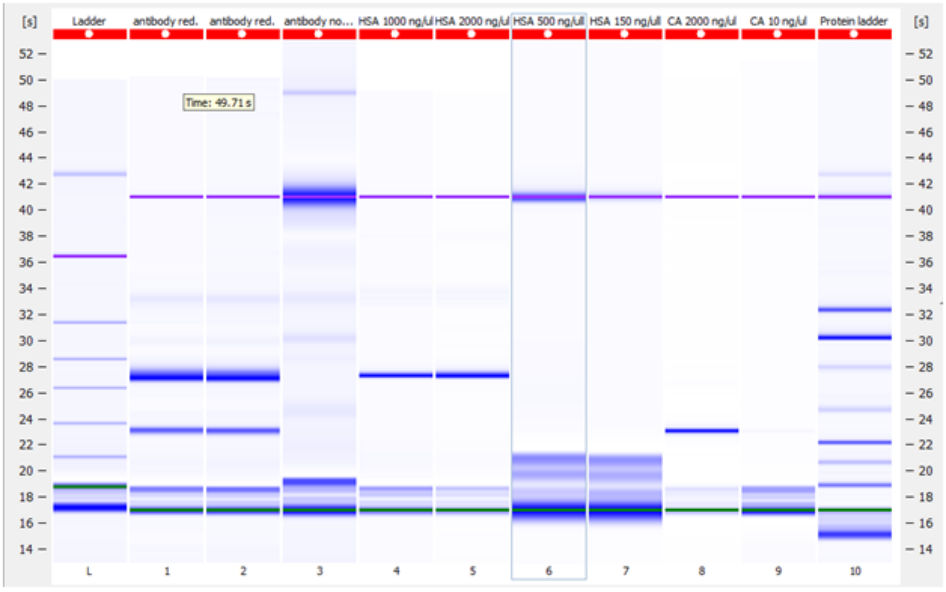


NOTE In the logbook, an error will appear: **Run aborted on port x. Instrument error occurred on port x, Unusual high or low voltage or current was detected during the start phase of the on-chip analysis.** The marked wells will indicate the wells on the chip that caused the problem.

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 126.
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 139. 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 30. If the power supply is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Unexpected Run Time

Unexpected Run Time

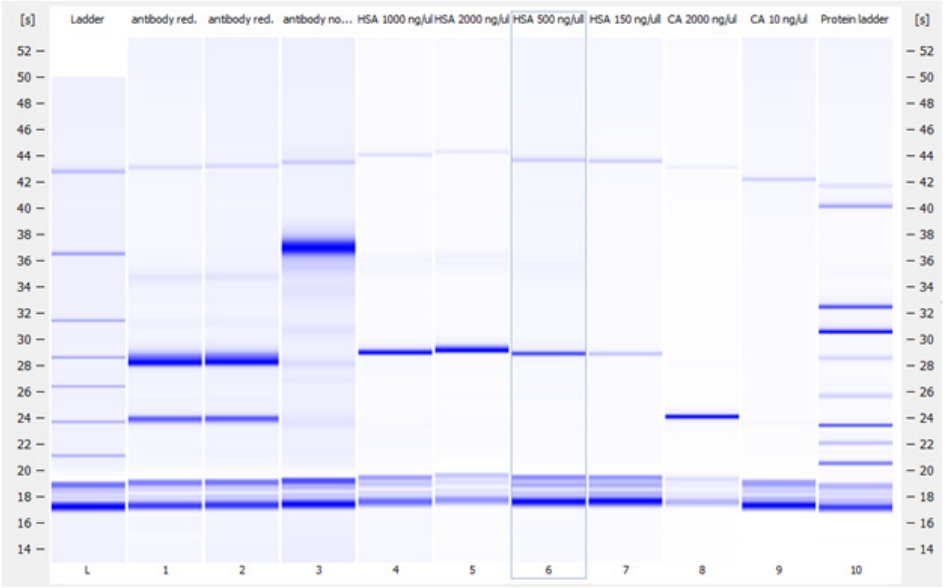


Most probable causes	*Solution*
Lower and/or upper markers are called incorrectly.	Turn off alignment and check which bands are the correct lower and upper markers. For more details see "Manual Marker Assignment" on page 96 .
Least probable causes	*Solution*
Bent electrode pin.	Check if electrode pins are bent or damaged. Replace electrophoresis cartridge.

Back to "Symptoms (Protein)" on page 89

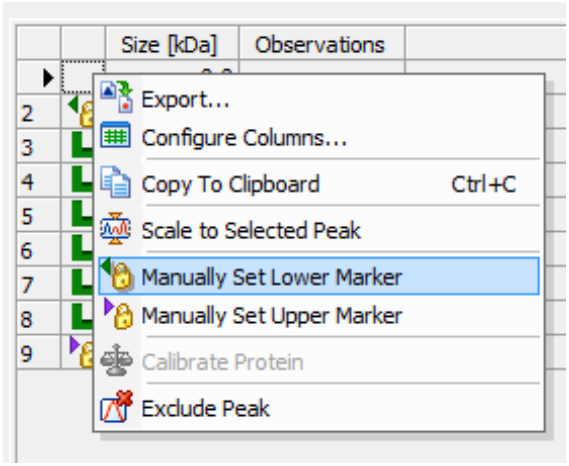
Manual Marker Assignment

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

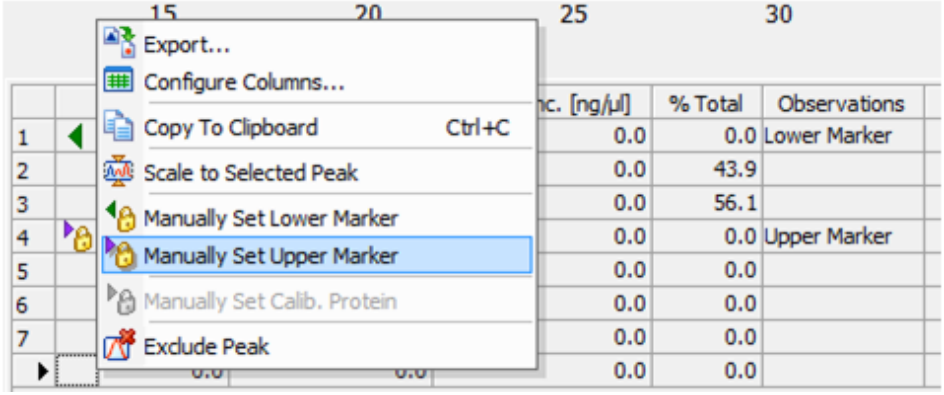


2 Turn the alignment back on  . Check the electropherogram of each sample. Go to the **Peak Table** tab to adjust the markers.

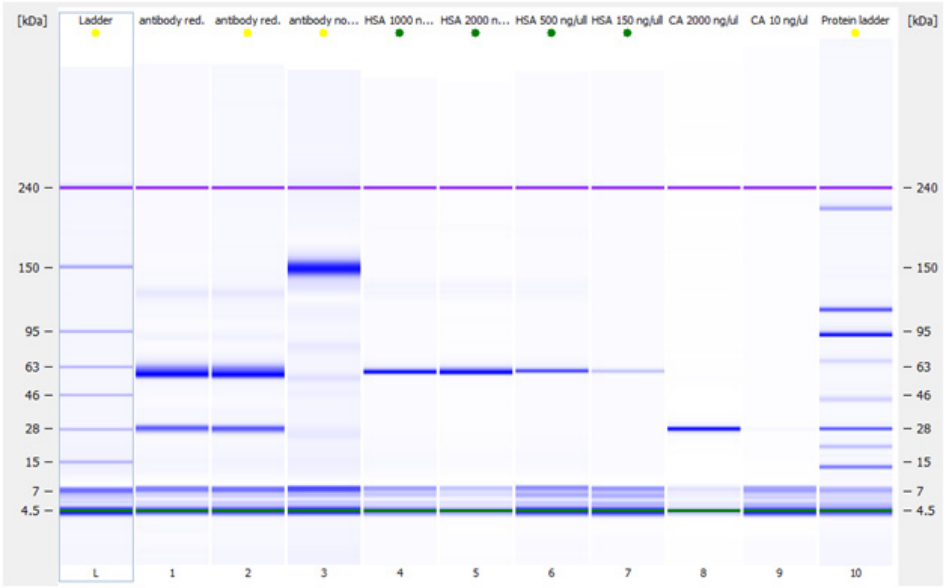
3 To adjust the lower marker, right click on the correct peak, and choose **Manually Set Lower Marker**.



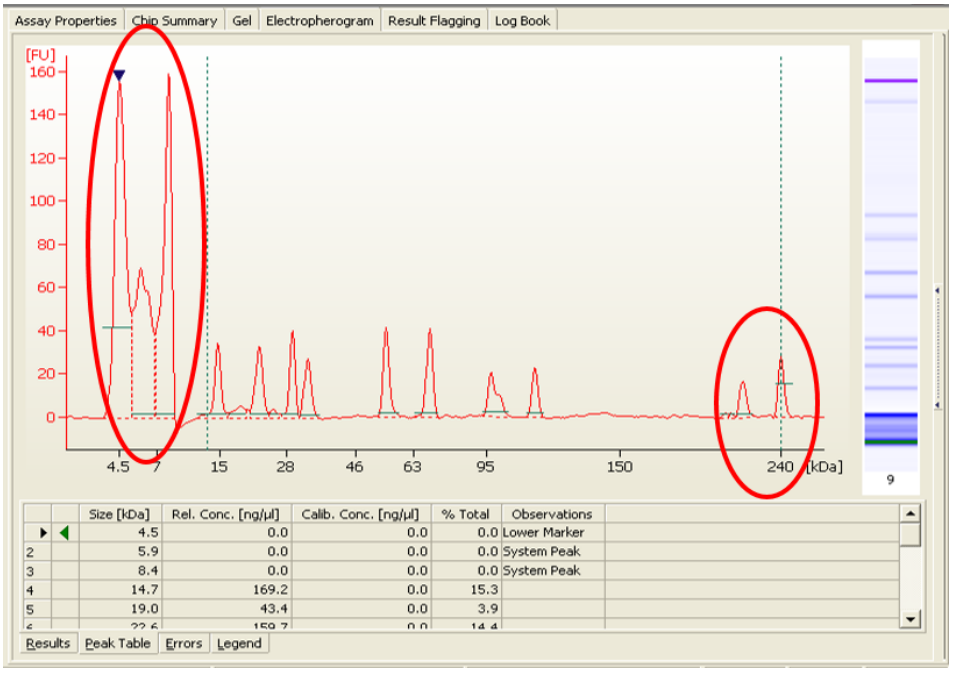
4 To adjust the upper marker, right click on the correct peak, and choose **Manually Set Upper Marker**.



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


[kDa]
Ladder
antibody red.
antibody red.
antibody no...
HSA 1000 n...
HSA 2000 n...
HSA 500 ng/ul
HSA 150 ng/ul
CA 2000 ng/ul
CA 10 ng/ul
Protein ladder
[kDa]
240
150
95
63
46
28
15
7
4.5
L
1
2
3
4
5
6
7
8
9
10

Additional Sample or Ladder Peaks

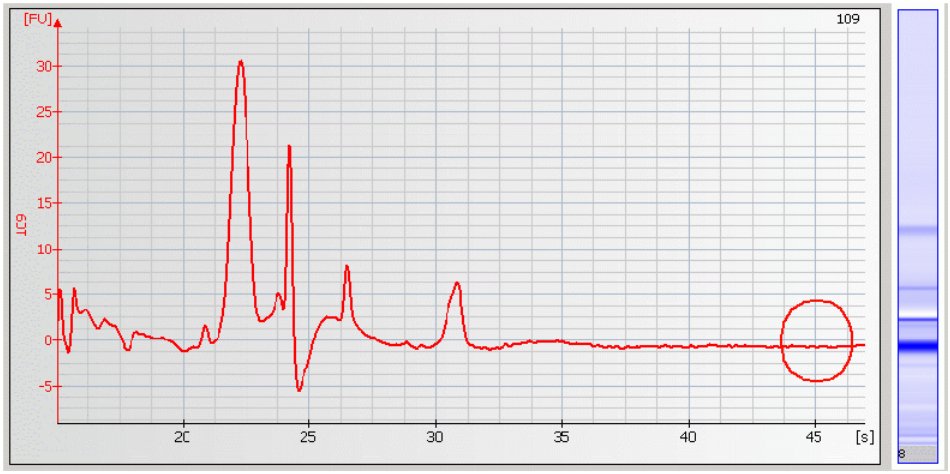


Most probable causes	*Solution*
Sample or ladder not denatured properly.	Prepare fresh sample aliquot. Heat sample or denaturing solution and ladder for 5 minutes at 100°C
Improper tubes used for denaturing samples.	Use 0.5 mL tubes for denaturing sample or denaturing solution.
Chip or gel-dye mix contaminated 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 126. 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.

Back to "Symptoms (Protein)" on page 89

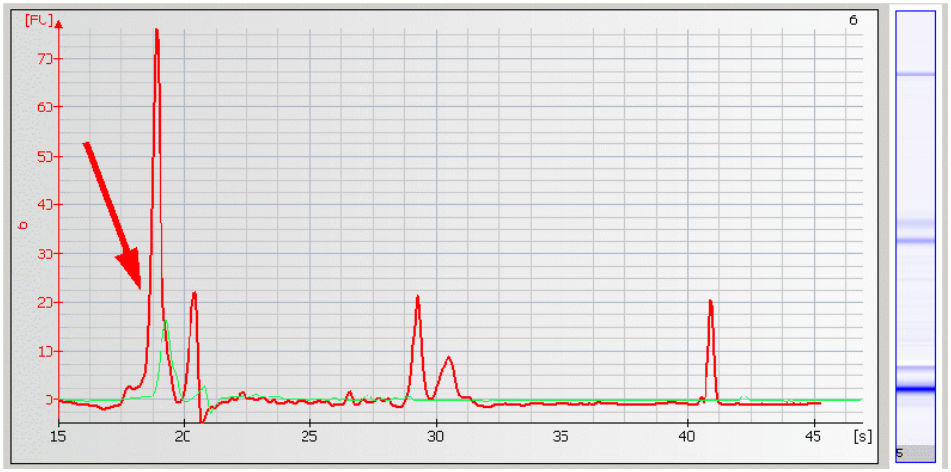
Missing Upper Marker



Most probable causes	*Solution*
Upper marker was called incorrectly.	Check upper marker assignment. Follow instructions for "Manual Marker Assignment" on page 96.
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, refer to the web site www.agilent.com/genomics/bioanalyzer. 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.

Back to "Symptoms (Protein)" on page 89

Broad Variability of the Lower Marker

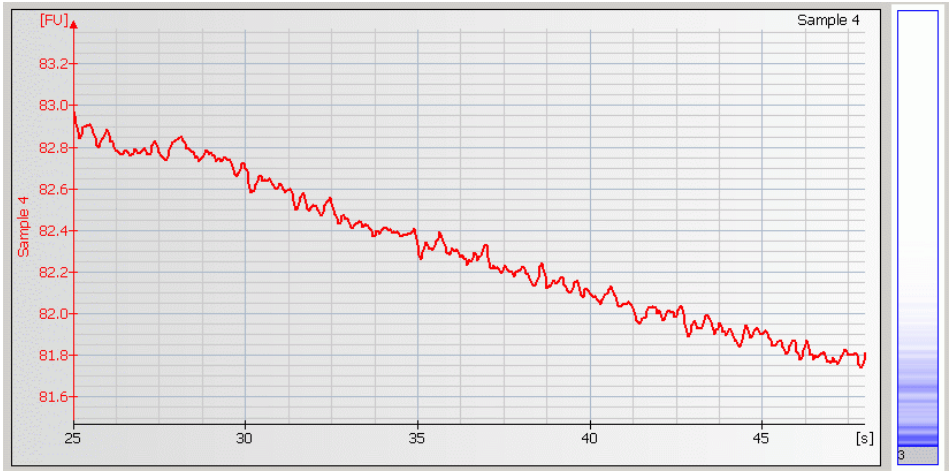


NOTE If the lower marker is detected, the assay performance is not affected by lower marker or system peak variability.

Most probable causes	*Solution*
Buffer components of the sample , e.g. salts, detergents, other additives etc. may interfere with the lower marker.	Ionic 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, refer to the web site www.agilent.com/genomics/bioanalyzer. 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.

Back to "Symptoms (Protein)" on page 89

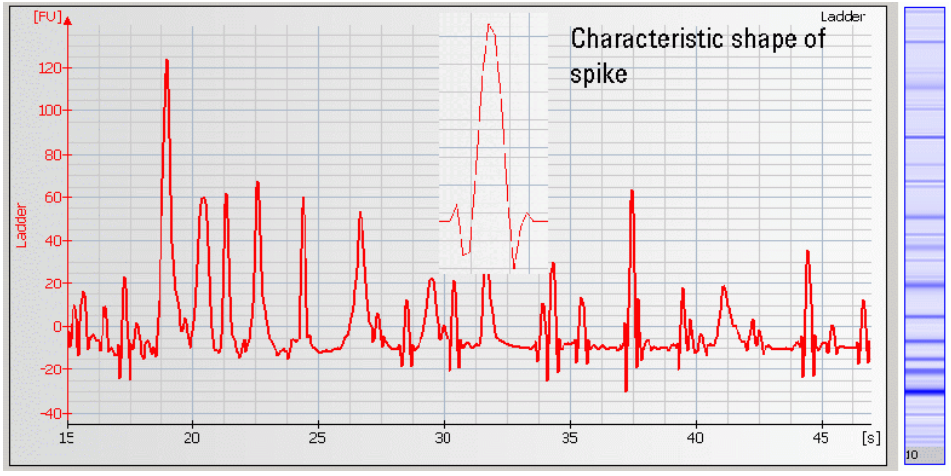
Missing Peaks



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 143. Do not touch the underside of the chip.
Least probable causes	*Solution*
Defective laser.	Check the laser stability using the "Overview" on page 30. If the laser test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.
Autofocus failure.	Check autofocus using the "Overview" on page 30. If autofocus test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.
High voltage power supply defective.	Check high voltage stability using the "Overview" on page 30. If the high voltage stability test fails, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (Protein)" on page 89

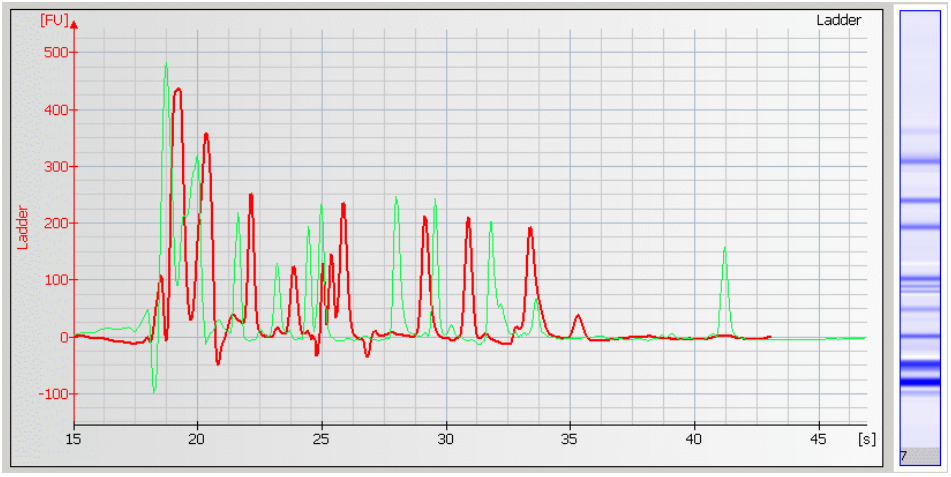
Spikes



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 115. 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 89

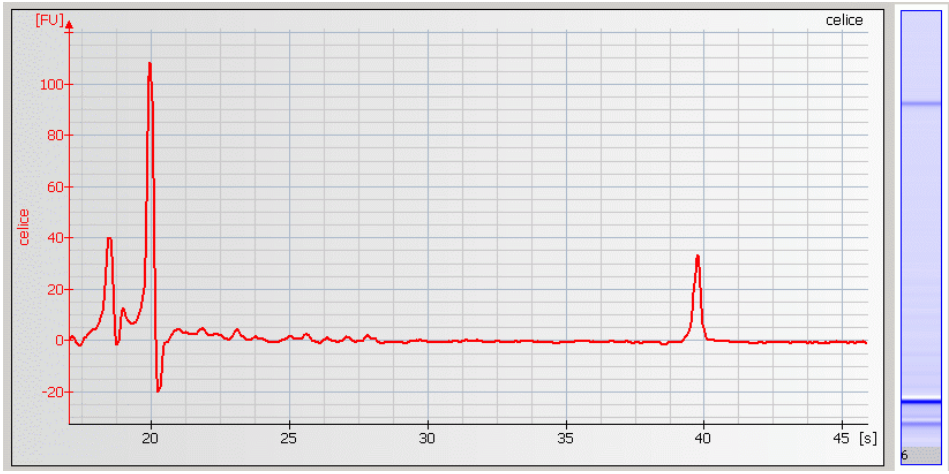
Poor Reproducibility



Most probable causes	*Solution*
Wrong peak alignment.	Check lower and upper marker assignment. Follow instructions for "Manual Marker Assignment" on page 96.
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 115.
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, refer to the web site www.agilent.com/genomics/bioanalyzer. If necessary, dilute, dialyze or desalt the sample.

Back to "Symptoms (Protein)" on page 89

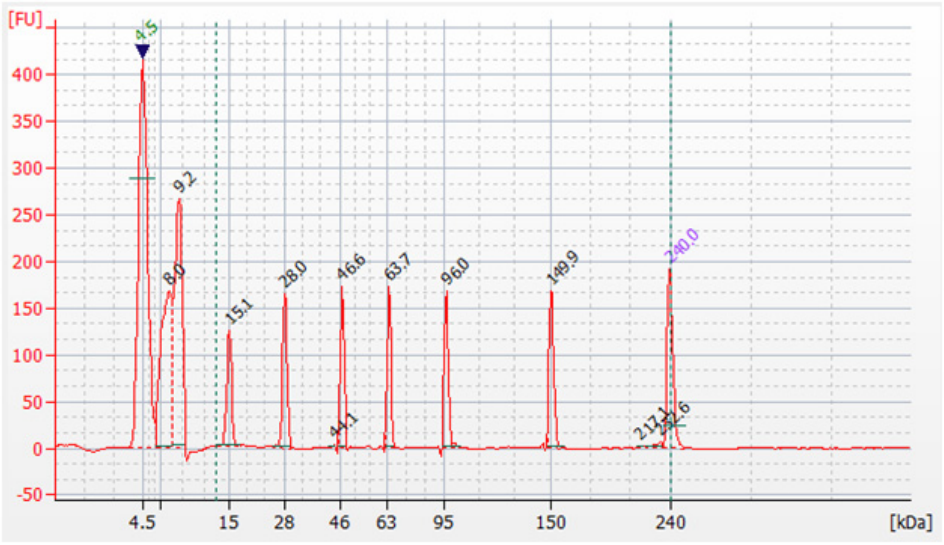
Low Signal Intensity



Most Probable Causes	*Solution*
Protein concentration is too low.	Follow specifications given in the Kit Guide.
Sample salt concentration is too high.	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.
Insufficient dissolution of SDS in the dye.	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.
Samples were not diluted prior to chip loading	Dilute samples according to protocol given in the Kit Guide.
Probable Causes	*Solution*
Improper denaturation of samples.	Prepare fresh sample aliquot. Heat sample and denaturating solution for 5 minutes at 100°C. Use 0.5 mL tubes for denaturation.
Pipetting error during preparation of reagent mixes.	Check dilution procedure and check calibration of pipette(s).
Least Probable Causes	*Solution*
Samples dissolved in acidic buffer.	Neutralize samples with appropriate buffer or dilute samples in deionized H_2O. Alternatively, dialyze samples against buffer with medium pH.

Back to “Symptoms (Protein)” on page 89

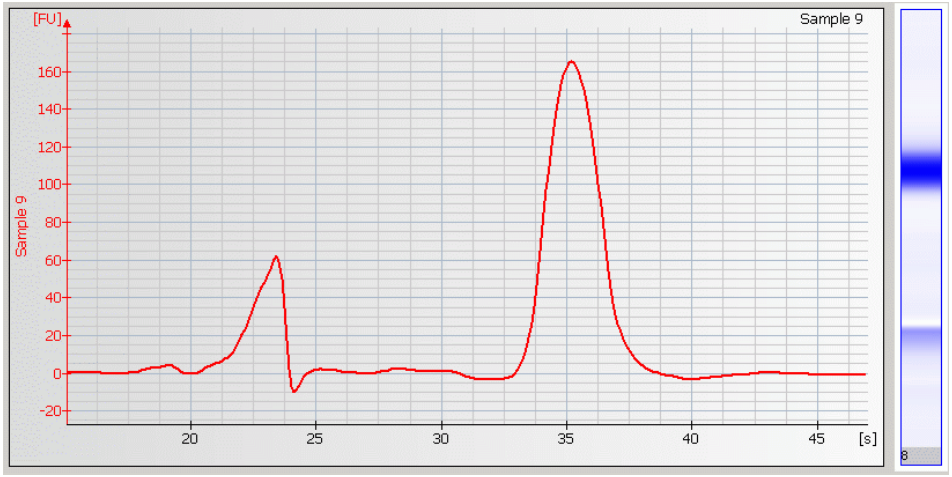
Low Ladder Peaks



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 89

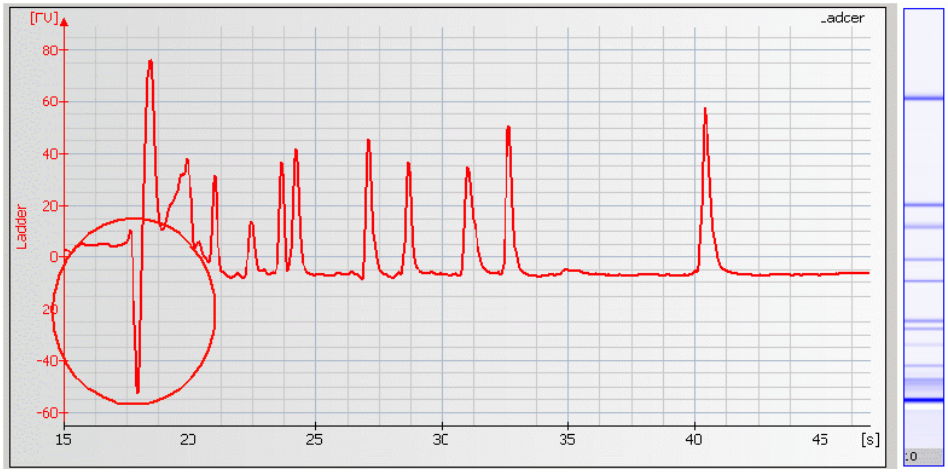
Broad Peaks



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 96.
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 139. 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 126. 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.

Back to "Symptoms (Protein)" on page 89

Baseline Dips

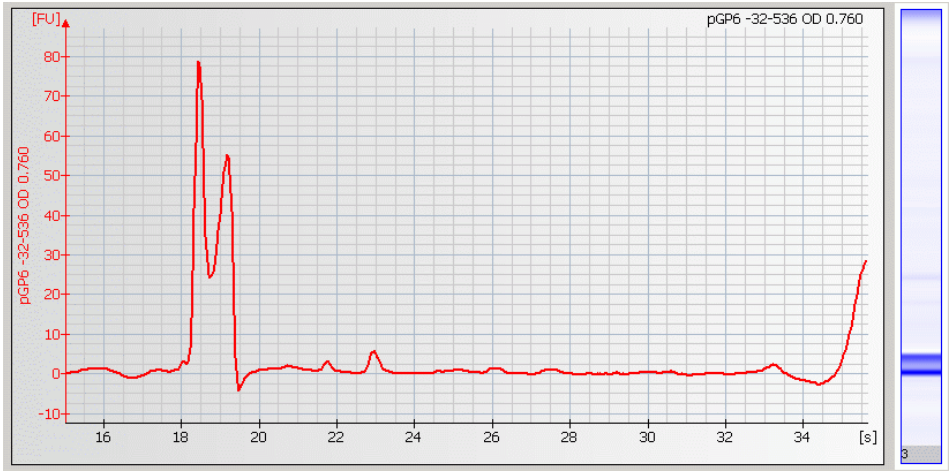


NOTE If the lower marker is detected, the assay performance is not affected by dips.

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, refer to the web site www.agilent.com/genomics/bioanalyzer. 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.

Back to "Symptoms (Protein)" on page 89

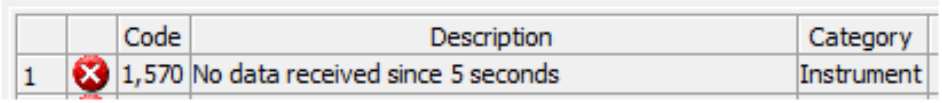
Late Migration



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 139. 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 accorting to specifications given in the Kit Guide.
Least probable causes	*Solution*
Defective heater plate.	Run the temperature test by using the "Overview" on page 30. If the heater plate is defective, contact Agilent Technologies at www.agilent.com/genomics/contact.

Back to "Symptoms (Protein)" on page 89

Error Message: No data received since 5 seconds



		Code	Description	Category
1		1,570	No data received since 5 seconds	Instrument

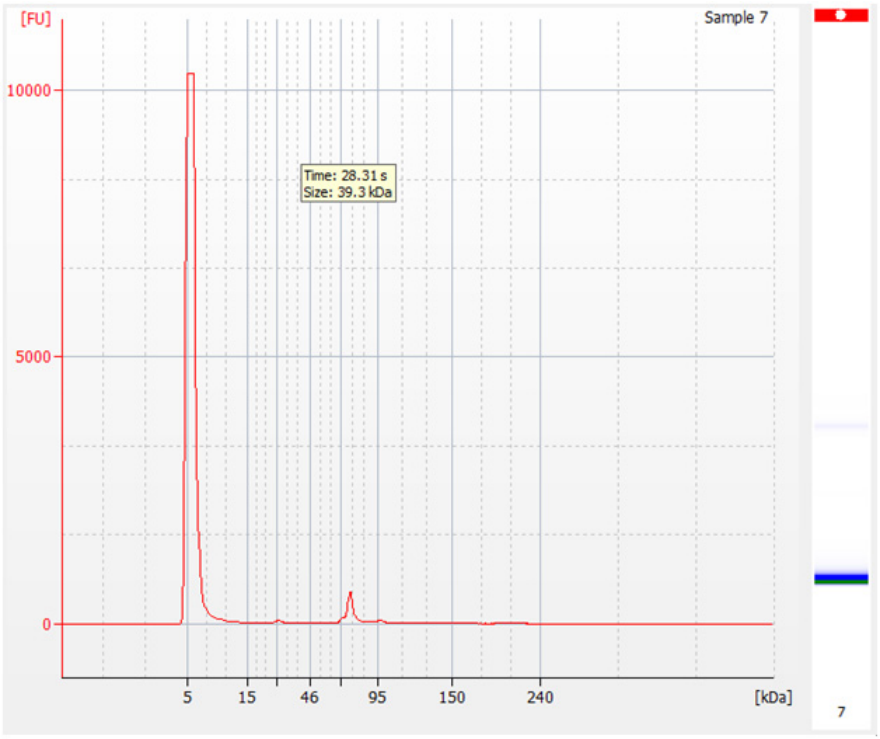
Most probable causes	*Solution*
Disrupted communication between instrument and computer.	Please refer to "Troubleshooting the Instrument Communication" on page 15 for troubleshooting instrument communication issue. Ensure the Agilent USB-Serial Adapter cable, black cable (5188-8031) for 2100 Expert Software 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 21.

Symptoms (High Sensitivity Protein)

Click to go straight to the troubleshooting hints.

-  "Saturation of Lower Marker or Sample Peaks – Optical Signal too High" on page 113
-  "Low Signal Intensity" on page 114

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

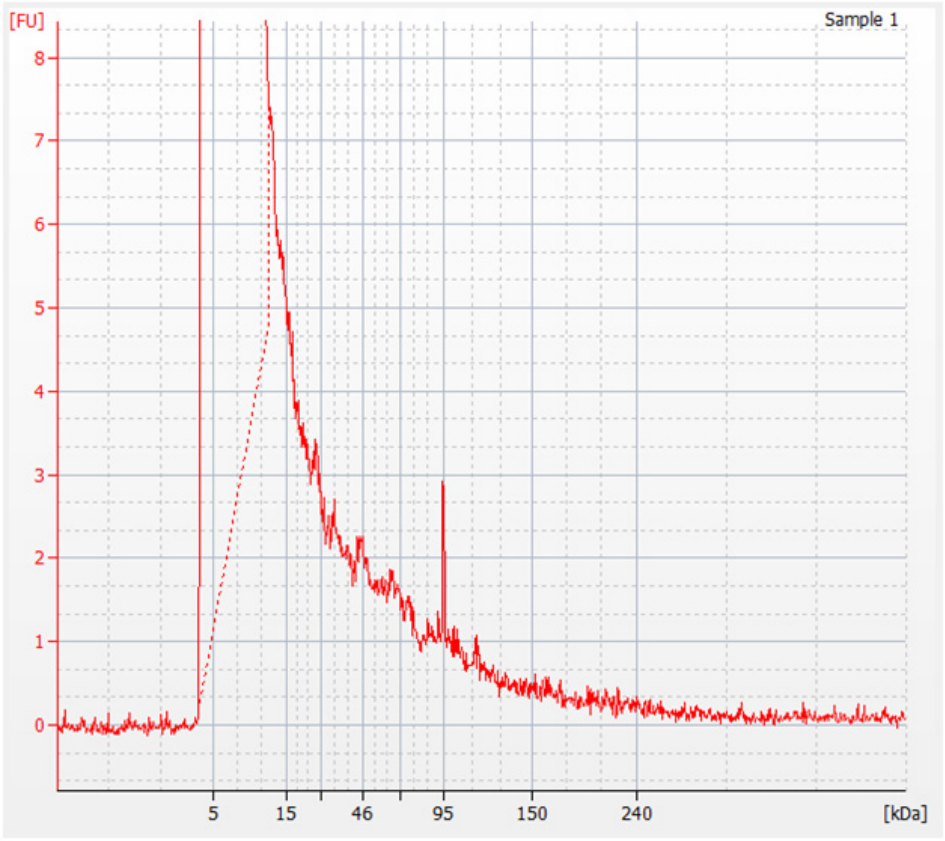


		Code	Description	Category
1	⊗	559	Optical signal too high (1605h)	Instrument

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 112

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. Low abundant proteins may require pico labeling (Technical Note 5990-3703EN).

Back to "Symptoms (High Sensitivity Protein)" on page 112

8
Maintenance of the Electrode Cartridge

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 126. Table 4 on page 116 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_2O for 10 seconds.	Electrode cleaner: deionized H_2O for 10 seconds.	With brush: deionized H_2O or isopropanol.
RNA Nano	Electrode cleaner: • RNaseZAP for 60 seconds. • RNase free H_2O for 10 seconds.	Electrode cleaner: • RNase free H_2O for 10 seconds.	RNase decontamination with brush: • RNaseZAP. • RNase free H_2O.
RNA Pico and Small RNA	Electrode cleaner: • RNase free H_2O for 5 minutes.	Electrode cleaner: • RNase free H_2O for 30 seconds.	RNase decontamination with brush: • RNaseZAP • RNase free H_2O

NOTE Electrode cleaner should be filled with 350 µL of solution (H_20 or RNaseZAP).

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

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_2O for 10 seconds.	Electrode cleaner: • deionized H_2O for 10 seconds.	With brush: • deionized H_2O or isopropanol

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 µL 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 126.

RNA Nano Assay

Assay	Before each run	After each run	Monthly or after liquid spill: Pin set cleaning
RNA Nano	Electrode cleaner: • RNase ZAP for 60 seconds. • RNase free H_2O for 10 seconds.	Electrode cleaner: • RNase free H_2O for 10 seconds.	RNase decontamination with brush: • RNase ZAP. • RNase free H_2O.

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.

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 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 µL 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.

NOTE 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 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 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 126.

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	Electrode cleaner: • RNase free H_2O for 5 minutes.	Electrode cleaner: • RNase free H_2O for 30 seconds.	RNase decontamination with brush: • RNaseZAP • RNase free H_2O

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

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

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 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.

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 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 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.

NOTE 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 126.

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 127. When the lever is in the vertical position, the cartridge is released from the lid by about 10 mm.

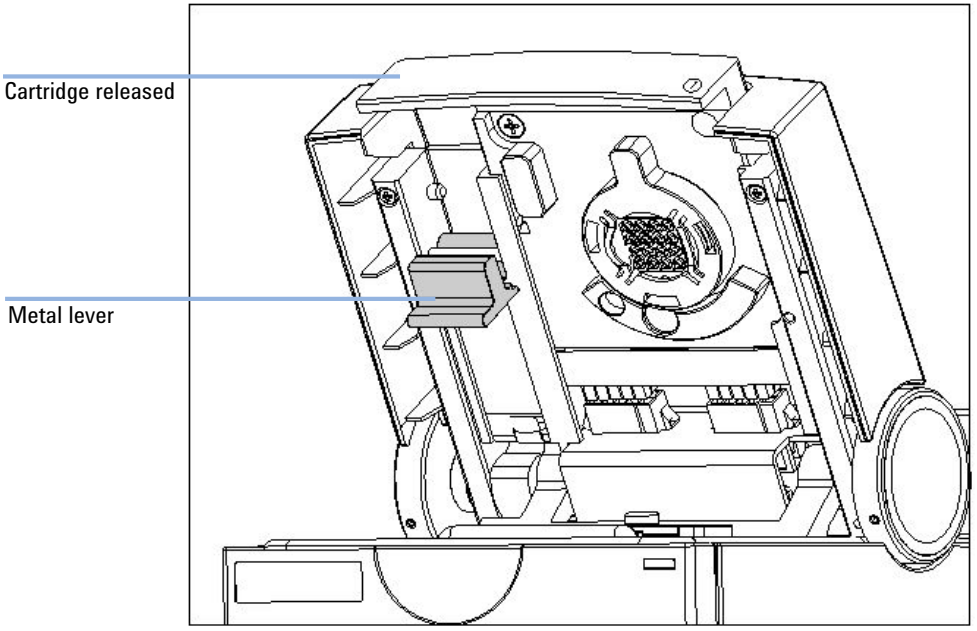


Figure 1 Remove/replace the electrode cartridge

3 Gently pull the cartridge out of the lid as shown in Figure 1 on page 127.

4 Open the bayonet socket of the pin set by turning the plastic lever to the left, see Figure 2 on page 128.

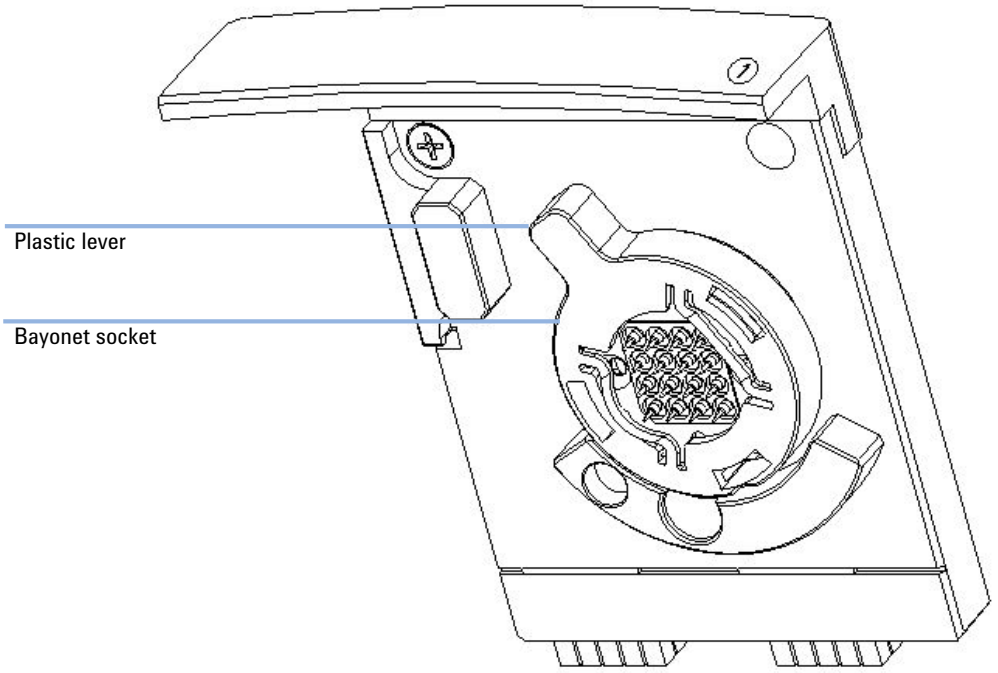


Figure 2 Bayonet socket of the electrode pin set

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 129.

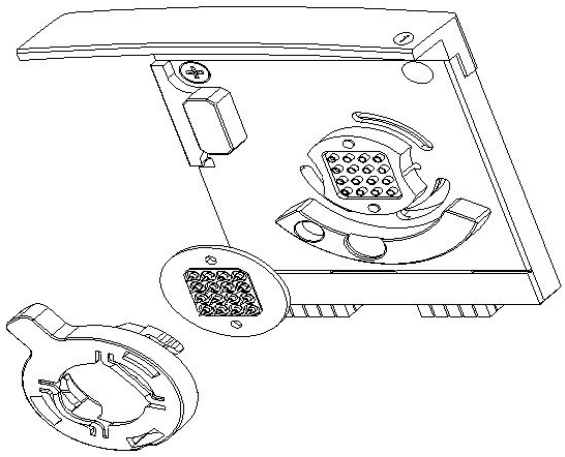


Figure 3 Bayonet cover and pin set

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 129.

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 128.

12 Slide the electrode cartridge with the pin set into the 2100 Bioanalyzer instrument lid as shown in Figure 1 on page 127 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 127.

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

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 133.
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 136.
Replacing the gasket	Every 3 months	...gasket is damaged, torn or contaminated with dried gel, see "Replacing the Gasket" on page 137.
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 139.

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 139.

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 134. The ring will come off.

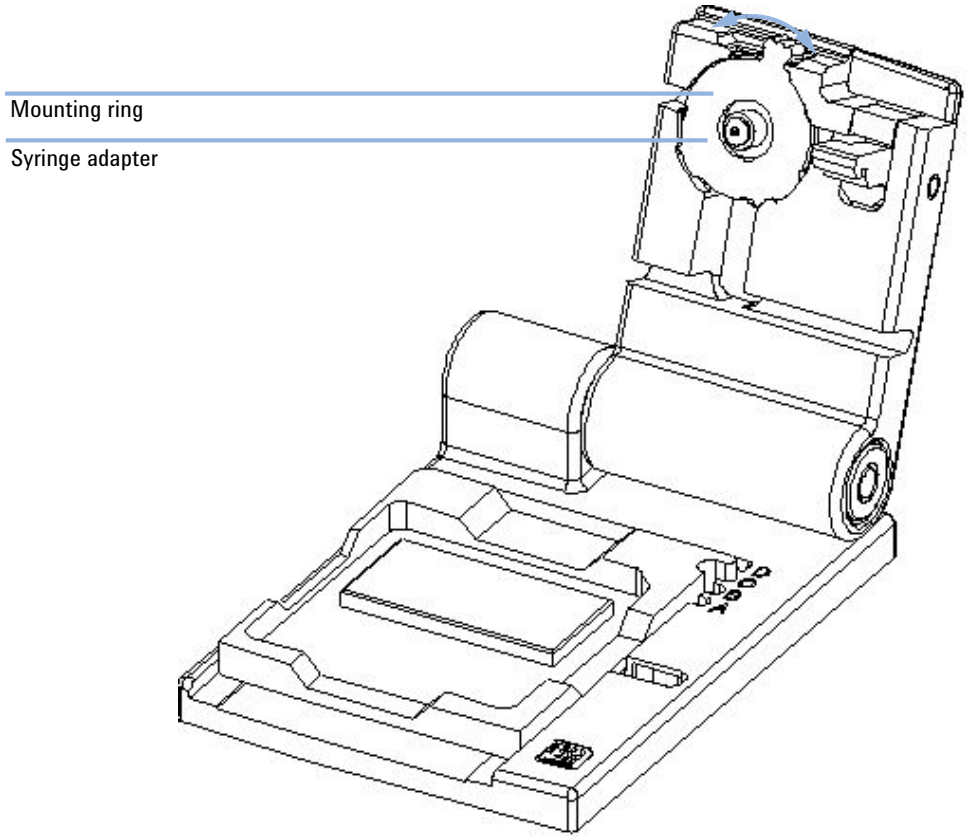


Figure 4 Mounting ring of the syringe adapter

3 Press the syringe adapter out of its mount as shown in Figure 5 on page 135.

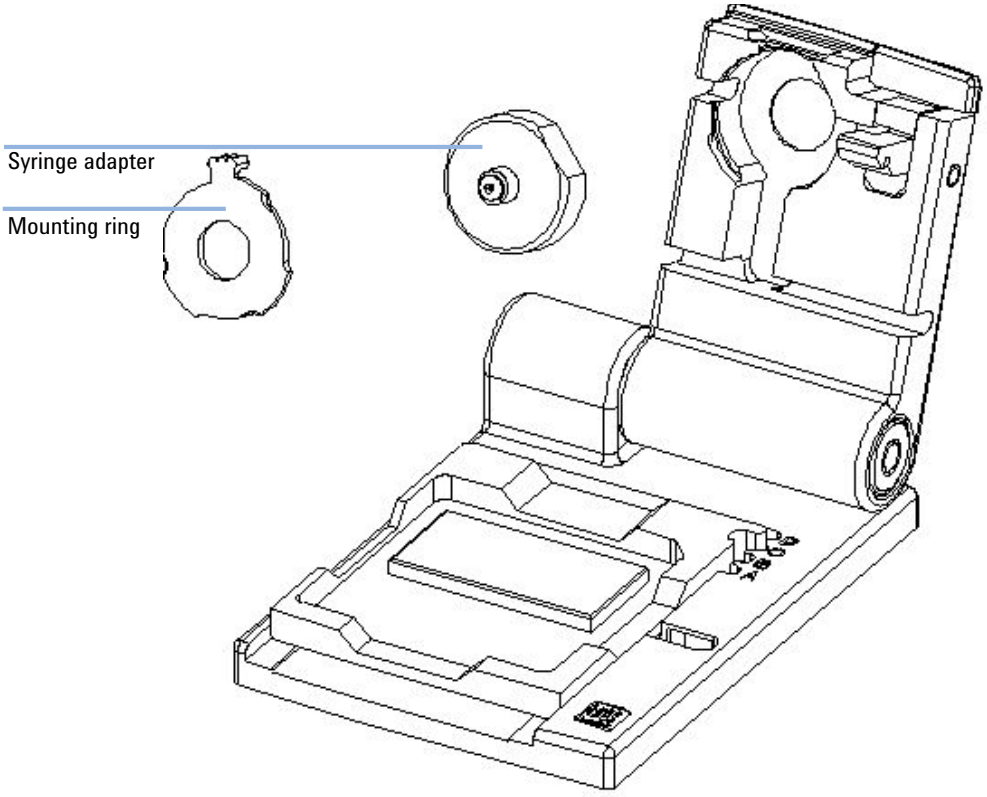


Figure 5 Removing/replacing the syringe adapter

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 135.

9 Follow the steps as described in "Cleaning the Syringe Adapter" on page 134 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 139.

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 134 to remove the syringe adapter.
2. Dispose the old syringe adapter.
3. Insert the syringe adapter, see Figure 5 on page 135.
4. Follow the steps as described in "Cleaning the Syringe Adapter" on page 134 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 139.

Replacing the Gasket

The silicone gasket, see Figure 6 on page 137, ensures a tight connetion between the chip and syringe adapter.

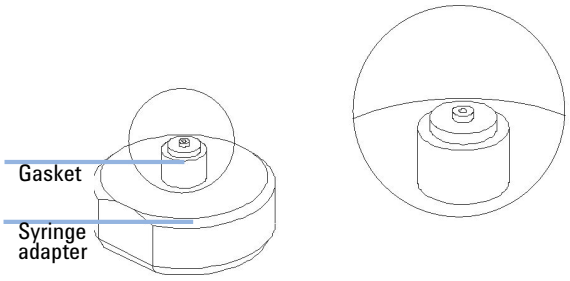


Figure 6 Syringe adapter with gasket

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 136.

2 Pull out the old silicone gasket with your fingers or tweezers. See Figure 7 on page 138 for a disassembled adapter.

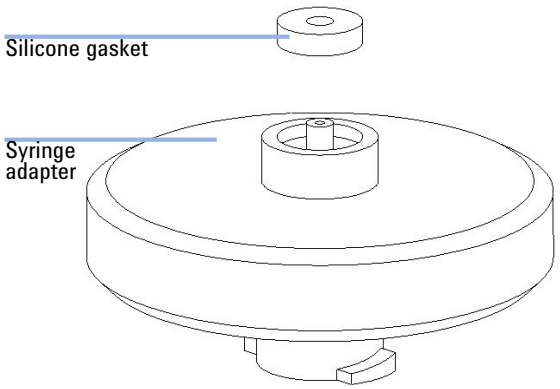


Figure 7 Syringe adapter with disassembled gasket

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 136 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 139.

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 139.

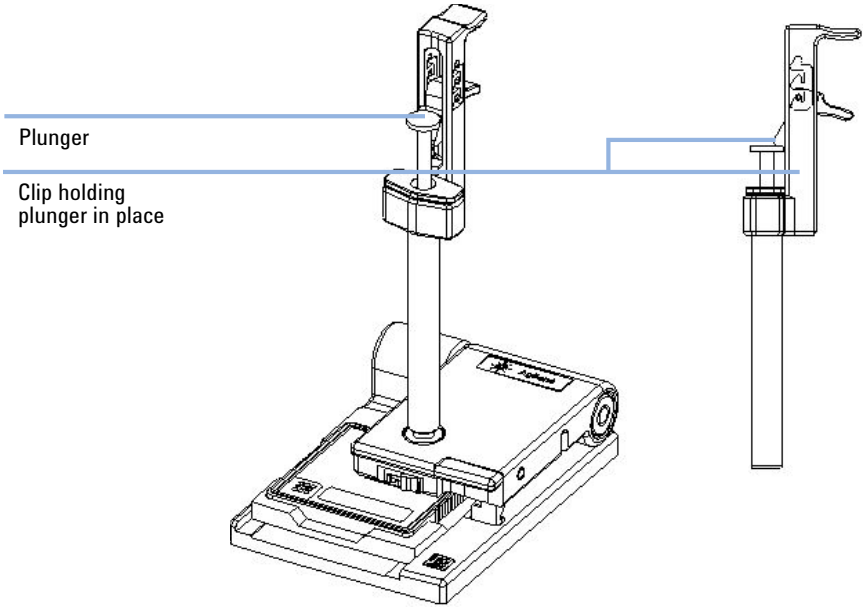


Figure 8 Locking the plunger of the syringe with the clip

6 Wait for 5 seconds and lower latch of the clip to release the plunger as shown in Figure 9 on page 140.

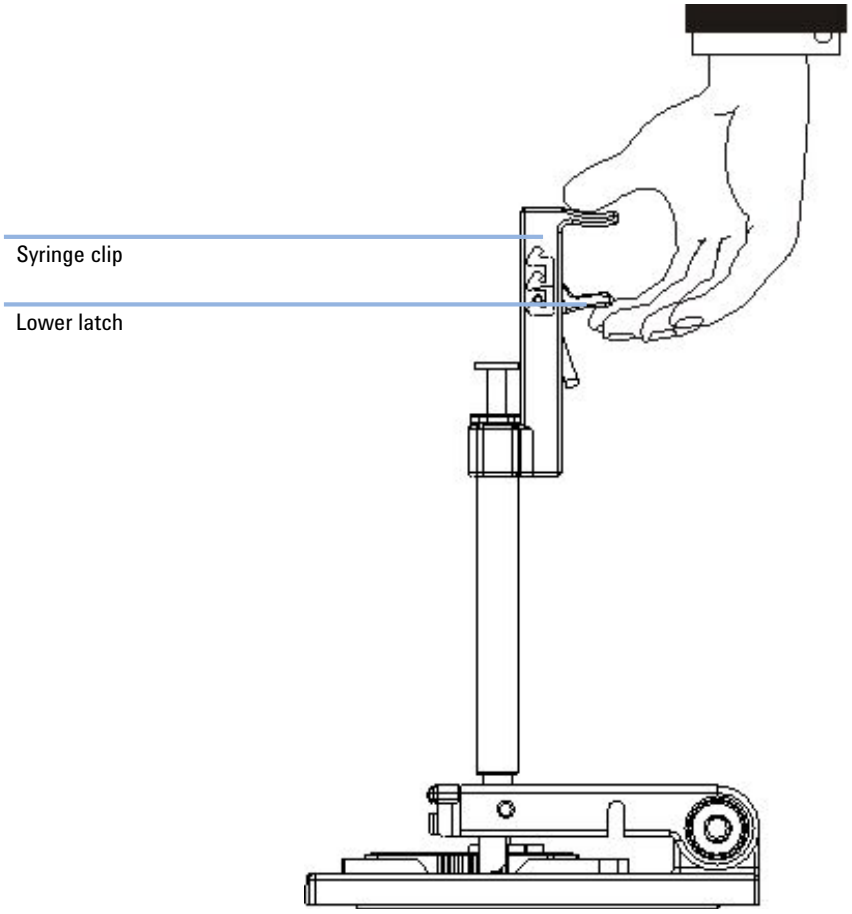


Figure 9 Releasing the plunger from the syringe

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

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 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 143	latest every 3 months	...lens is contaminated with liquid spill or noticeably dirty
"Changing the Fuses" on page 144	n/a	...status indicator is off and the cooling fan is not running

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.

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 145.

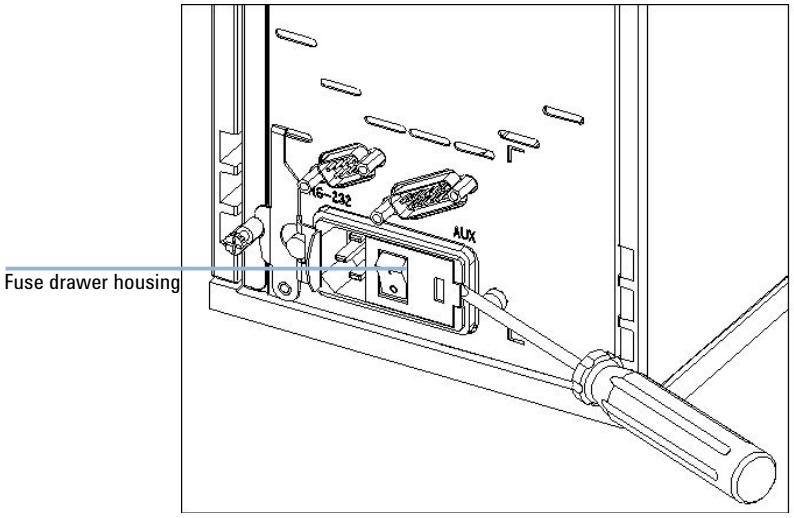


Figure 10 Remove power inlet housing

4 Pull out the fuse drawer as shown in Figure 11 on page 145.

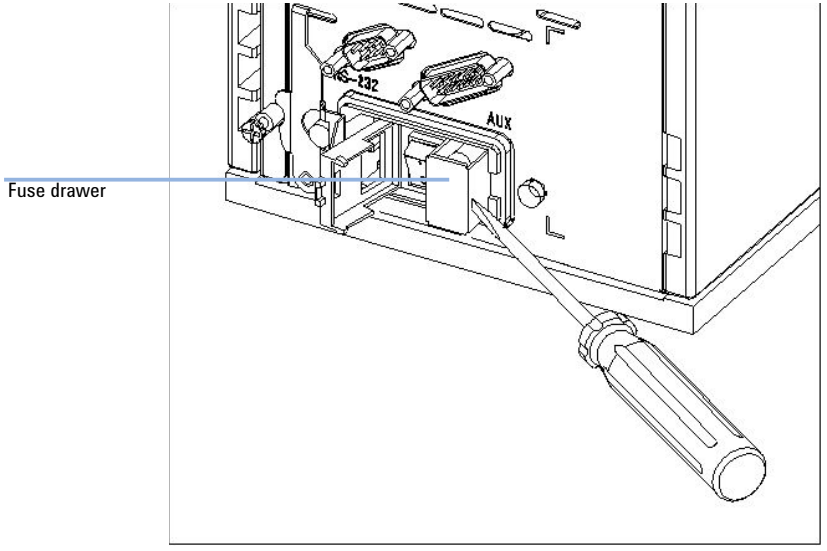


Figure 11 Remove fuse drawer

5 Replace the two fuses.

NOTE Perform this procedure with care.

6 Slide in the fuse drawer and push till it fits tightly as shown in Figure 12 on page 146.

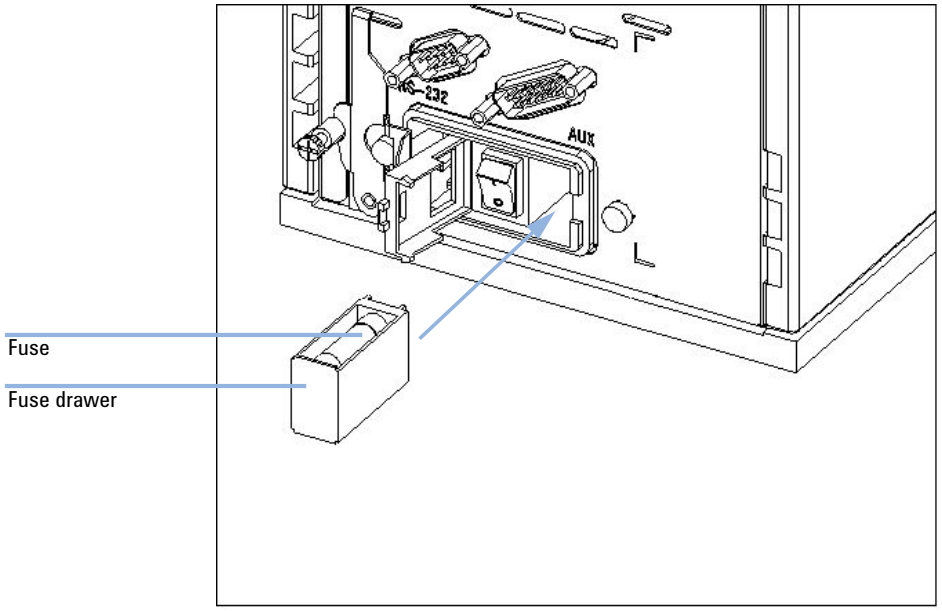


Figure 12 Insert fuse drawer

7 Close the fuse drawer housing (see Figure 13 on page 147), reconnect the instrument to the power line and switch it on.

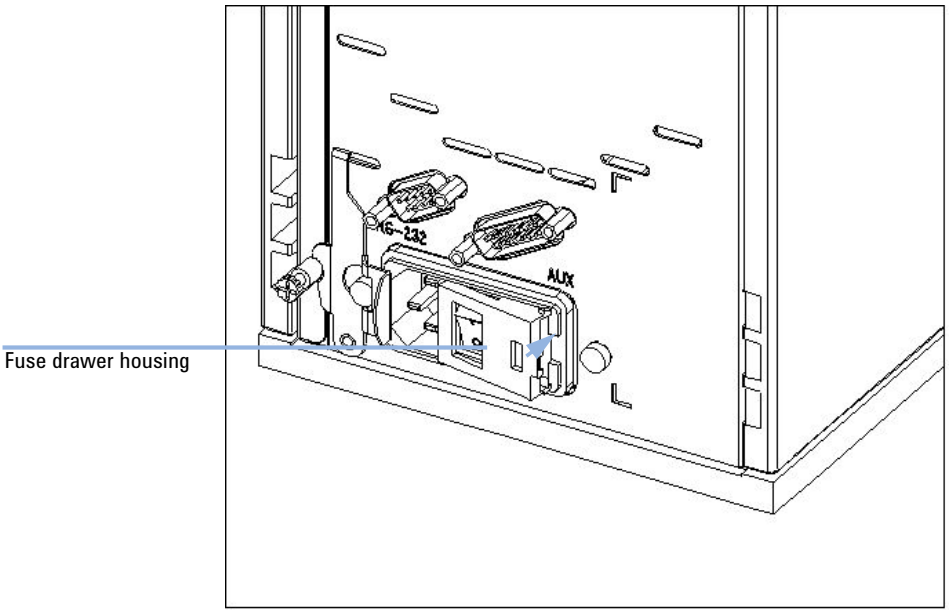


Figure 13 Close fuse drawer housing

11
Maintenance of the Vortexer

Changing the Adapter

When Whenever the vortex adapter is damaged.

Tools required

p/n	Description
NA	Screw driver

Parts required

#	p/n	Description
1	5065-9966	IKA vortex mixer adapter (for MS 2 vortexers)
1	IKA 3617036	IKA vortex mixer adapter (for MS 3 vortexers) NOTE: This part must be purchased directly from IKA.

1. Release the 3 or 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 3 or 4 screws with the screw driver.

12
Spare Parts and Accessories

Overview

"Overview" on page 152 provides a list of spare parts and accessories that are available for the 2100 Bioanalyzer system.

To buy parts, please refer to the Agilent Online Store: www.agilent.com/genomics/bioanalyzer

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.
5065-9966	Vortex mixer adapter	For IKA MS2 vortexer.
IKA 3617036	Vortex mixer adapter	For IKA MS3 vortexer (must be ordered through IKA).

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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. Other software revisions may have an impact on results.

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