Small RNA Kit for 2100 Bioanalyzer Systems

Quick Guide

The complete *Small RNA Kit for 2100 Bioanalyzer Systems Kit Guide* can be found in the online help of the Agilent 2100 Expert software.

Kit Components

Agilent Small RNA Kit (5067-1548)	
Agilent Small RNA Chips	**Agilent Small RNA Reagents (5067-1549) & Supplies**
25 Small RNA Chips	● (blue) Small RNA Dye Concentrate (1 vial)
3 Electrode Cleaners	● (green) Small RNA Marker (4 vials)
	○ (white) Small RNA Conditioning Solution
Syringe Kit	● (red) Small RNA Gel Matrix (2 vials)
1 Syringe	● (yellow) Small RNA Ladder (1 vial) (5067-1550)
	2 Spin Filters (5185-5990) + 30 tubes for gel-dye mix
Tubes for Gel-Dye Mix	
30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free) for gel-dye mix	

For Research Use Only

Not for use in Diagnostic Procedures.

Assay Principles

Agilent RNA kits contain chips and reagents designed for analysis of RNA fragments. Each RNA chip contains an interconnected set of microchannels that is used for separation of nucleic acid fragments based on their size as they are driven through it electrophoretically. Agilent RNA kits are designed for use with the 2100 Bioanalyzer system only.

Applications and Kits

Agilent Small RNA kits are designed for the analysis of total RNA (eukaryotic and prokaryotic) and samples enriched for small nucleic acids.
Available kits: Agilent RNA 6000 Nano kit (5067-1511), RNA 6000 Pico kit (5067-1513), and Small RNA kit (5067-1548).

Storage Conditions

- Freeze unopened Small RNA ladder at -28 – -15 °C (-18 – 5 °F). Prepared ladder aliquots need to be stored at -28 – -15 °C (-18 – 5 °F). Keep all other reagents and reagent mixes refrigerated at 2 – 8 °C (36 – 46 °F) when not in use to avoid poor results caused by reagent decomposition.

- Protect marker solution (contains a fluorescent dye), dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Store the chips at room temperature.

Equipment Supplied with the Agilent 2100 Bioanalyzer System

- Chip priming station (5065-4401)
- IKA vortex mixer

Additional Material Required (Not Supplied)

- RNaseZAP® recommended for electrode decontamination *only*
- RNase-free water, recommended for routine electrode cleaning
- Pipettes (10 µL and 1000 µL) with compatible tips (RNase-free, no filter tips, no autoclaved tips)
- 0.5 mL and 1.5 mL microcentrifuge tubes (RNase-free Eppendorf Safe-lock PCR clean or Eppendorf DNA LoBind microcentrifuge tubes are highly recommended)
- Microcentrifuge (> 13000 g)
- Heating block or water bath for ladder/sample preparation
- Mandatory: Dedicated bayonet electrode cartridge (5065-4413) for RNA 6000 Pico and Small RNA assays
- 2100 Expert SW rev B.02.05 or higher

Sample Preparation

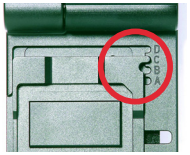
- Prepare RNA samples in deionized water. For analysis, the concentration of total RNA should be between 1 – 100 ng/µL, purified small RNA samples (< 150 nt) should be 1 – 20 ng/µL, and oligonucleotides should be 100 – 2000 pg/µL. If concentration of a sample is above these ranges, dilute with RNase-free water.

Specifications

Physical Specifications		Analytical Specifications	
Analysis time	30 min	Quantitative range	50 – 2000 pg/µL of purified miRNA in water
Samples per chip	11	Qualitative range	50 – 2000 pg/µL of purified miRNA in water
Sample volume	1 µL	Size range	6 – 150 nt
Kit stability	4 months	Sensitivity (S/N>3)	50 pg/µL in water
Kit size	25 chips 11 samples/chip = 275 samples/kit	Quantitative precision (within a chip)	25 % CV
		Quantitative accuracy	-
		Maximum buffer concentration in sample	10 mM Tris 0.1 mM EDTA

Setting up the Chip Priming Station

1 Replace the syringe:
- **a** Unscrew the old syringe from the lid of the chip priming station.
- **b** Release the old syringe from the clip. Discard the old syringe.
- **c** Remove the plastic cap of the new syringe and insert it into the clip.
- **d** Slide it into the hole of the luer lock adapter and screw it tightly to the chip priming station.

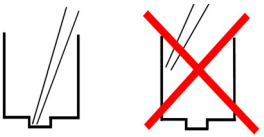


2 Adjust the base plate:
- **a** Open the chip priming station by pulling the latch.
- **b** Using a screwdriver, open the screw at the underside of the base plate.
- **c** Lift the base plate and insert it again in position C. Retighten the screw.

3 Adjust the syringe clip:
   a Release the lever of the clip and slide it down to the lowest position.

Essential Measurement Practices

- Handle and store all reagents according to the instructions on the label of the individual box.
- Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.
- Allow all reagents to equilibrate to room temperature for 30 min before use. Thaw the sample on ice.
- Protect marker solution, dye and dye mixtures from light. Remove light covers only when pipetting. The dye decomposes when exposed to light and this reduces the signal intensity.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results.
- Always wear gloves when handling RNA and use RNase-free tips, microfuge tubes and water.
- The gel matrix is very viscous! Proper pipetting and mixing requires special attention.
- It is recommended to heat denature all RNA samples and RNA ladder before use for 2 min at 70 °C (once) and keep them on ice.
- Do not touch the 2100 Bioanalyzer instrument during analysis and never place it on a vibrating surface.
- Always vortex the dye concentrate for 10 s before preparing the gel-dye mix and spin down afterwards.
- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 min after preparation. Reagents might evaporate, leading to poor results.
- To prevent contamination (e.g RNase), it is strongly recommended to use a dedicated electrode cartridge for RNA assays.
- For cleaning of the electrode pinset inbetween runs, use RNase-free water only. Do not use RNaseZAP unless for decontamination of the electrode pins. After using RNaseZAP, wash the pins thoroughly with RNase-free water. Refer to the kit guide for details on electrode cleaning and decontamination.



Agilent Small RNA Assay Protocol

WARNING

Handling Reagents

The dye can cause eye irritation. Because the dye binds to nucleic acids, it should be treated as a potential mutagen.

Kit components contain DMSO. DMSO is skin-permeable and can elevate the permeability of other substances through the skin.

- ✓ **Follow the appropriate safety procedures and wear personal protective equipment including protective gloves and clothes as well as eye protection.**
- ✓ **Follow good laboratory practices when preparing and handling reagents and samples.**
- ✓ **Always use reagents with appropriate care.**
- ✓ **For more information, refer to the material safety data sheet (MSDS) on www.agilent.com.**

Preparing the RNA Ladder

1 Spin the ladder down.
2 Heat denature the ladder for 2 min at 70 °C.
3 Immediately cool the vial on ice.
4 Prepare aliquots in recommended 0.5 mL RNase-free vials with the required amount for typical daily use.
5 Store aliquots at -28 – -15 °C (-18 – 5 °F). After initial heat denaturation, the frozen aliquots should not require repeated heat denaturation.
6 Before use, thaw ladder aliquots on ice (avoid extensive warming).

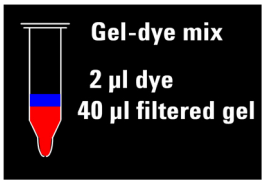
Preparing the Gel

1 Pipette the complete volume (approx. 650 µL) of the gel matrix (red ●) into a spin filter.
2 Centrifuge at 10000 g ± 20 % for 15 min at room temperature. Use filtered gel within 4 weeks. Store at 2 – 8 °C (36 – 46 °F).

Small RNA Kit

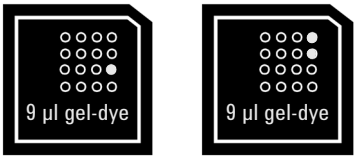
Preparing the Gel-Dye Mix

1 Allow all reagents to equilibrate to room temperature for 30 min.
2 Vortex dye concentrate (blue ●) for 10 s and spin down.
3 Pipette 2 µL of dye into 0.5 mL RNase-free microtubes (provided with the kit).
4 Add 40 µL of filtered gel (gel is very viscous, careful pipetting is required).
5 Mix using pipetting, or by flipping over and flicking the vial several times until the dye is distributed equally. Visually inspect that the mix is homogeneous.
6 Spin tube at 13000 g for 10 min at room temperature.



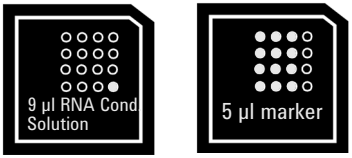
Loading the Gel-Dye Mix

1 Put a new Small RNA chip on the chip priming station.
2 Pipette 9 µL of gel-dye mix in the well marked G.
3 Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
4 Press plunger until it is held by the clip.
5 Wait for exactly 60 s then release clip.
6 Visually inspect that the plunger moves back at least to the 0.3 mL mark.
7 Wait for 5 s, then slowly pull back the plunger to the 1 mL position.
8 Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.
9 Discard the remaining gel-dye mix.



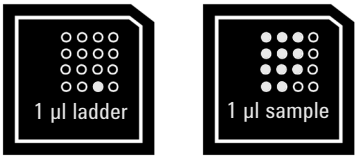
Loading the Conditioning Solution and Marker

1 Pipette 9 µL of the RNA conditioning solution (white ○) into the well marked CS.
2 Pipette 5 µL of RNA marker (green ●) in all 11 sample wells and in the well marked ⚷.



Loading the Ladder and Samples

1 Pipette 1 µL of prepared ladder in well marked ⚷.
2 Pipette 1 µL of sample in each of the 11 sample wells. Pipette 1 µL of RNA marker (green ●) in each unused sample well.
3 Put the chip horizontally in the IKA vortexer and vortex for 1 min at 2400 rpm.
4 Run the chip in the 2100 Bioanalyzer instrument within 5 min.



Technical Support

Please visit our support web page **www.agilent.com/genomics/contactus** to find information on your local Contact Center.

Further Information

Visit the Agilent website. It offers useful information, support, and current developments about the products and technology: **www.agilent.com/en/product/automated-electrophoresis/bioanalyzer-systems**.

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