Agilent RNA 6000 Pico Kit Quick Start Guide

The complete RNA 6000 Pico Guide can be found in the online help of the 2100 Expert software.

Agilent RNA 6000 Pico Kit (reorder-no 5067-1513)	
Agilent RNA 6000 Pico Chips	*Agilent RNA 6000 Pico Reagents (reorder-no 5067-1514) & Supplies*
25 RNA Pico Chips	● (blue) RNA 6000 Pico Dye Concentrate[1]
3 Electrode Cleaners	● (green) RNA 6000 Pico Marker (4 vials)
	○ (white) RNA 6000 Pico Conditioning Solution
Syringe Kit	● (red) RNA 6000 Pico Gel Matrix (2 vials)
1 Syringe	● (yellow) RNA 6000 Pico Ladder (reorder-no 5067-1535) (1 vial, 10x concentrate)
	4 Spin Filters (reorder-no 5185-5990)
Tubes for Gel-Dye Mix	
30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free) for gel-dye mix	

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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 Agilent 2100 Bioanalyzer instrument only.

Applications and Kits

Agilent RNA kits are designed for the analysis of total RNA (eukaryotic, prokaryotic, and plant) and mRNA samples.

Agilent RNA kits: RNA 6000 Nano Kit (reorder-no 5067-1511), RNA 6000 Pico Kit (reorder-no 5067-1513), and Small RNA Kit (reorder-no 5067-1548)

Storage Conditions

- Freeze unopened RNA ladder at -20 °C. Prepared ladder aliquots need to be stored at -70 °C. Keep all other reagents and reagent mixes refrigerated at 4 °C when not in use to avoid poor results caused by reagent decomposition.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Agilent Technologies

Equipment Supplied with the Agilent 2100 Bioanalyzer System

- Chip priming station (reorder-no 5065-4401)
- IKA vortex mixer

Additional Material Required (Not Supplied)

- RNaseZAP® recommended for electrode decontamination *only* (Ambion, Inc. cat. no. 9780)
- 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 (reorder-no 5065-4413) for RNA 6000 Pico and Small RNA assays.

Sample Preparation

- Prepare RNA samples in deionized water. For estimation of RNA concentration, the total RNA concentration in the sample must be between 200 – 5000 pg/µL. The mRNA concentration must be between 500 – 5000 pg/µL.If concentration of a sample is above this range, dilute with RNase-free water.

Specifications

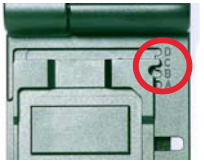
Physical Specifications		Analytical Specifications		
Type	**Specification**	**Specification**	**Total RNA Assay**	**mRNA Assay**
Analysis run time	30 min	Qualitative range	50–5000 pg/µL (in water) (Signal/Noise>3)	250–5000 pg/µL (in water) (Signal/Noise>3)
Number of samples	11 samples/chip	Quantitation reproducibility	20 % CV	20 % CV
Sample volume	1 µL	Quantitation accuracy	30 % CV (for ladder as sample)	30 % CV (for ladder as sample)
Kit stability	4 months (Storage temperature see individual box!)	Buffer compatibility[1]	50 mM Tris or 50 mM NaCl	50 mM Tris or 50 mM NaCl

[1] Due to the high sensitivity of the assay, different ions and higher salt concentrations might influence the performance of the assay. It is recommended to analyze the samples in water, as this will yield the highest sensitivity.

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:

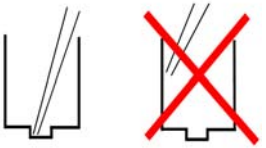
- **a** Open the chip priming station by pulling the latch.
- **b** Using a screwdriver, open the screw at the underside of the base plate.
- **c** Lift the base plate and insert it again in position C. Retighten the screw.

3 Adjust the syringe clip:

a Release the lever of the clip and slide it up to the top 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 samples on ice.
- Protect 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, microcentrifuge tubes and water.
- It is recommended to heat denature all RNA samples and RNA ladder before use for 2 min and 70 °C (once) and keep them on ice.
- Do not touch the Agilent 2100 Bioanalyzer instrument during analysis and never place it on 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 RNA 6000 Pico Assay Protocol

WARNING

Handling DMSO

Kit components contain DMSO. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

→ Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

→ Handle solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Preparing the RNA Ladder

1 Spin the ladder down and pipette in an RNase-free vial.

2 Heat denature the ladder for 2 min at 70 °C.

3 Immediately cool the vial on ice.

4 Add 90 µL of RNase-free water and mix thoroughly.

5 Prepare aliquots in recommended 0.5 mL RNase-free vials with the required amount for typical daily use.

6 Store aliquots at -70 °C. After initial heat denaturation, the frozen aliquots should not require repeated heat denaturation.

7 Before use, thaw ladder aliquots on ice (avoid extensive warming).

Preparing the Gel

1 Pipette 550 µL of RNA gel matrix (red ●) into a spin filter.
2 Centrifuge at 1500 g ± 20 % for 10 min at room temperature.
3 Aliquot 65 µL filtered gel into 0.5 mL RNase-free microcentrifuge tubes. Use filtered gel within 4 weeks. Store at 4 °C.

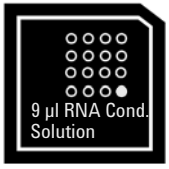
Preparing the Gel-Dye Mix

1 Allow the RNA dye concentrate (blue ●) to equilibrate to room temperature for 30 min.
2 Vortex RNA dye concentrate (blue ●) for 10 s, spin down and add 1 µL of dye into a 65 µL aliquot of filtered gel.
3 Vortex solution well. Spin tube at 13000 g for 10 min at room temperature. Use prepared gel-dye mix within one day.

Loading the Gel-Dye Mix

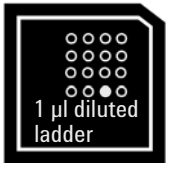
1 Put a new RNA chip on the chip priming station.
2 Pipette 9 µL of gel-dye mix in the well marked G.
3 Make sure that the plunger is positioned at 1 mL and then close the chip priming station.
4 Press plunger until it is held by the clip.
5 Wait for exactly 30 s then release clip.
6 Wait for 5 s. Slowly pull back plunger to 1 mL position.
7 Open the chip priming station and pipette 9 µL of gel-dye mix in the wells marked G.
8 Discard the remaining gel-dye mix.

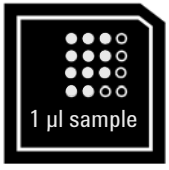
Loading the Conditioning Solution and Marker



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 Diluted Ladder and Samples





1 Pipette 1 µL of the heat denatured and aliquoted ladder in the 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 Agilent 2100 Bioanalyzer instrument within 5 min.

Technical Support

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

Further Information

Visit the 2100 Bioanalyzer site at http://www.agilent.com/genomics/bioanalyzer. You can find useful information, support and current developments about the products and the technology.

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