Agilent RNA 6000 Pico Kit Quick Start Guide

Agilent RNA 6000 Pico Kit (reorder number 5067-1513)	
RNA Chips	**Reagents (reorder number 5067-1514) & Supplies**
25 RNA Pico Chips	(blue) RNA 6000 Pico Dye Concentrate*
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 number 5067-1535) (1 vial, 10x concentrate)
	4 Spin Filters + 30 tubes for gel-dye mix

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Assay Principles

Agilent RNA kits contain chips and reagents designed for analysis of RNA fragments. Each RNA Pico chip contains an interconnected set of microchannels that 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 only.

Assay Kits

Agilent RNA kits are designed for the analysis of total RNA (eukaryotic and prokaryotic) and mRNA samples. The complete RNA 6000 Pico kit guide can be found in the online help of the 2100 expert software.

Other RNA kits: Agilent RNA 6000 Nano kit (reorder-no 5067-1511), Agilent Small RNA kit (reorder-no 5067-1548)

Storage Conditions

- Freeze the unused RNA 6000 ladder at -20°C and keep all 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.

Equipment Supplied with the Agilent 2100 Bioanalyzer

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

Additional Material Required (Not Supplied)

- RNaseZAP® recommended for electrode decontamination (Ambion, Inc. cat. no. 9780)
- RNase-free water
- Pipettes (10 µl and 1000 µl) with compatible tips (RNase-free)
- 0.5 ml and 1.5 ml microcentrifuge tubes (RNase-free)
- Microcentrifuge (≥ 1300g)
- Heating block or water bath for ladder/sample preparation
- Mandatory: bayonet electrode cartridge (reorder number 5065-4413)

Sample Preparation

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

Physical Specifications

Type	Specification
Analysis run time	30 minutes
Number of samples	11 samples/chip
Sample volume	1 µl
Kit stability	4 months (Storage temperature see individual box!)

Analytical Specifications

Specification	Total RNA Assay	mRNA Assay
Qualitative range	50–5000 pg/µl (in water) (Signal/Noise>3)	250–5000 pg/µl (in water) (Signal/Noise>3)
Reproducibility of quantitation	20 % CV	20 % CV
Quantitation accuracy	30 % CV (for ladder as sample)	30 % CV (for ladder as sample)
Buffer compatibility*	50 mM Tris or 50 mM NaCl	50 mM Tris or 50 mM NaCl

*)Due to the high sensitivity of the assay, different ions and higher salt concentrations might influence the performance of the assay.

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.

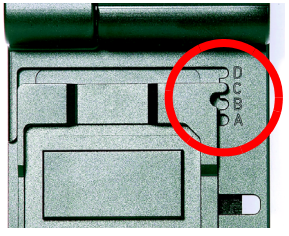
d Slide it into the hole of the luer lock adapter and screw it tightly to the priming station.

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.

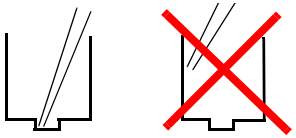


3 Adjust the syringe clip:

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 minutes before use.
- 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, microfuge tubes and water.
- It is recommended to heat denature all RNA samples and RNA ladder before use for 2 minutes and 70 °C (once) and keep them on ice (“Preparing the RNA ladder after arrival” on page 14 of the Kit guide).
- Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on vibrating surface.
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye mix.
- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 minutes after preparation. Reagents might evaporate, leading to poor results.
- To prevent contamination (e.g. RNase) problems, it is strongly recommended to use a dedicated electrode cartridge for RNA assays. For running the RNA 6000 Pico assay, the 16 pin bayonet cartridge is mandatory.
- Perform the RNase decontamination procedure for the electrodes daily before running any assays.

Agilent RNA 6000 Pico Assay Protocol - Edition April 2007

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

Preparing the RNA Ladder after Arrival

1 After reagent kit arrival, spin ladder down and transfer the entire volume (10 µl) to a RNase-free vial.

2 Heat denature it for 2 min at 70 °C.

3 Immediately cool down the vial on ice.

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

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

6 Store aliquots at -70 °C. After initial heat denaturing, the frozen aliquots needn't be heat denatured again.

7 Before use thaw ladder aliquots and keep them on ice (avoid extensive warming).

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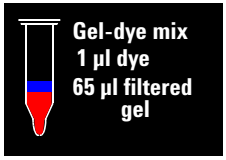
Preparing the Gel

1 Pipette 550 µl of RNA 6000 Pico gel matrix (red ●) into a spin filter.
2 Centrifuge at 1500 g ± 20 % for 10 minutes at room temperature.
3 Aliquot 65 µl filtered gel into 0.5 ml RNase-free microfuge tubes. Use filtered gel within 4 weeks.

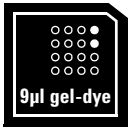
Preparing the Gel-Dye Mix

1 Allow the RNA 6000 Pico dye concentrate (blue ●) to equilibrate to room temperature for 30 min.
2 Vortex RNA 6000 Pico dye concentrate (blue ●) for 10 seconds, 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 6000 Pico chip on the chip priming station.
2 Pipette 9.0 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 seconds then release clip.
6 Wait for 5 s. Slowly pull back plunger to 1ml position.
7 Open the chip priming station and pipette 9.0 µl of gel-dye mix in the wells marked G.
8 Discard the remaining gel-dye mix.

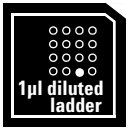


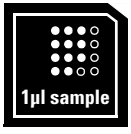
Loading the RNA 6000 Pico Conditioning Solution and Marker

1 Pipette 9.0 µl of the RNA 6000 Pico Conditioning Solution (white ❍) in the well marked CS.
2 Pipette 5 µl of RNA 6000 Pico 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 6000 Pico Marker (green ●) in each unused sample well.
3 Put the chip in the adapter of the IKA vortexerand vortex for 1 min at the indicated setting (2400 rpm).
4 Run the chip in the Agilent 2100 bioanalyzer within 5 min.





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Further Information Visit Agilent Technologies' unique Lab-on-a-Chip web site. It is offering useful information, support and current developments about the products and the technology: http://www.agilent.com/chem/labonachip.

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