RNA 6000 Pico Assay Quick Reference Guide

RNA 6000 Pico LabChip® Kit (reorder number 5065-4473)

RNA 6000 Pico Chips	RNA 6000 Reagents & Supplies
25 RNA Pico Chips	RNA 6000 Pico Dye Concentrate
3 Electrode Cleaners	RNA 6000 Pico Marker (4 vials)
	RNA 6000 Pico Conditioning Solution
Syringe Kit	RNA 6000 Pico Gel Matrix (2 vials)
1 Syringe	4 Spin Filters + 30 tubes for gel-dye mix

Assay Principles

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

Assay Kit

RNA LabChip® kits are designed for the analysis of total RNA (eukaryotic and prokaryotic) and messenger RNA samples.

Storage Conditions

- 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.

Accessory Products

- Chip Priming Station (reorder number 5065-4401)

Materials and Equipment

- Mandatory for the RNA Pico assay: Bayonet Electrode Cartridge (reorder number 5065-4413)
- Pipettes (10 µl and 1000 µl) with compatible tips (RNase free, filter tips recommended)
- Microcentrifuge and RNase free Microcentrifuge tubes: 0.5 and 1.5 ml
- IKA vortex mixer
- RNase free water
- RNA 6000 ladder (Ambion, Inc. cat. no. 7152)
- RNase ZAP® (Ambion, Inc. cat. no. 9780)

RNA 6000 Pico Physical Specifications

Type	Specification
Analysis run time	30 minutes
Number of samples	11 samples/chip
Sample volume	1 µl
Assay kit stability	3 months at 4 °C

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.

Decontamination the Electrodes (daily)

1. Fill an electrode cleaner with 350 µl RNase free water.
2. Place electrode cleaner in the Agilent 2100 bioanalyzer.
3. Close the lid and leave closed for 5 minutes.
4. Open the lid and remove the electrode cleaner.
5. Wait another 30 seconds for the water on the electrodes to evaporate.

Technical Support:

In the U.S./Canada	1-800-227-9770 (toll free)
	bioanalyzer_americas@agilent.com
In Europe	bioanalyzer_europe@agilent.com
In Japan	0120 477 111
	lab_chip@agilent.com
In Asia Pacific	(+81) 422 56 93 92
	bioanalyzer_ap@agilent.com

Further Information

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

Essential Measurement Practices

- *Always* insert the pipette tip into the bottom of the chip well when dispensing liquids. Placing the pipette at the edge of the well may lead to bubbles and poor results.
- Strictly follow the cleaning procdure. The RNA Pico assay is sensitive and any contaminations will disturb the analysis.
- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents to warm up to room temperature for 30 minutes before use.
- Protect dye and gel-dye mix from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Prepared chips must be used within 5 minutes. Reagents may evaporate, leading to poor results.
- Vortex chips for 1 minute at the appropriate setting (2400 rpm).
- Use a new syringe and electrode cleaners with each new LabChip Kit.
- Use RNase-free tips, microfuge tubes and water.

RNA 6000 Pico Analytical Specifications

Specification	Total RNA Assay	mRNA Assay
Qualitative range	200–5000 pg/µl	500–5000 pg/µl
Maximum sample buffer strength*	10 mM Tris-0.1 mM EDTA	10 mM Tris-0.1 mM EDTA

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

RNA 6000 Pico Assay Protocol - Edition November 2003

Preparing the Gel

1 Put 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.

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 Close Chip Priming Station.
4 Press plunger until it is held by the clip.
5 Wait for exactly 30 seconds then release clip.
6 Pipette 9.0 µl of gel-dye mix in the wells marked G.
7 Discard the remaining gel-dye mix.

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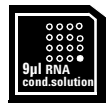
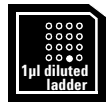
Loading the RNA 6000 Pico Conditioning Solution and Marker

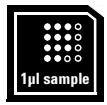
1 Pipette 9.0 µl of the RNA 6000 Pico Conditioning Solution (yellow ●) in the well marked CS.
2 Pipette 5 µl of RNA 6000 Pico marker (green ●) in all 11 sample wells and in the well marked ⌀.



Loading the diluted Ladder and Samples

1 Pipette 1 µl of diluted ladder in 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 and vortex for 1 min at the set-point of the IKA vortexer.
4 Run the chip in the Agilent 2100 bioanalyter within 5 min.





WARNING —Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. No data is available addressing the mutagenicity or toxicity of the dye/DMSO reagent. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling DMSO stock solutions.

Detach and keep as a reference with your instrument