RNA 6000 Nano Assay Quick Reference Guide

RNA 6000 Nano LabChip® Kit (reorder number 5065-4476)

RNA 6000 NanoChips

25 RNA Nano Chips

2 Electrode Cleaners

Syringe Kit

1 Syringe

RNA 6000 Nano Reagents & Supplies

- RNA Nano Dye Concentrate
- RNA 6000 Nano Marker (1 vial)
- RNA 6000 Nano Gel Matrix (2 vials)

4 spin filters + 30 tubes for gel-dye mix

RNA 6000 Nano Physical Specifications

Type	Specification
Analysis run time	30 minutes
Number of samples	12 samples/chip
Sample volume	1 µl
Assay kit stability	3 months at 4 °C

Assay Principles

RNA LabChip® kits contain chips and reagents designed for sizing and analysis of RNA fragments. Each RNA LabChip® 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. 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.
Other RNA Kits: RNA 6000 Pico kit (reorder-no 5065-4473)

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

- Pipettes (10 µl and 1000 µl) with compatible tips (RNase free, filter tips recommended)
- RNase free water
- Microcentrifuge and RNase free microcentrifuge tubes: 0.5 ml and 1.5 ml
- RNase free water
- Heating block or water bath
- RNA 6000 ladder (Ambion, Inc. cat. no. 7152)
- RNaseZAP (Ambion, Inc. cat. no. 9780)

Sample Preparation

For determination of RNA concentration, total RNA in sample must be between 25–500 ng/µl. If concentration of your particular sample is above this range, dilute with RNase-free water.

Decontamination of the Electrodes (daily)

1. Fill an electrode cleaner with 350 µl RNaseZAP.
2. Place electrode cleaner in the Agilent 2100 bioanalyzer.
3. Close the lid and leave closed for 1 minute.
4. Open the lid and remove the electrode cleaner.
5. Fill *another* electrode cleaner with 350 µl RNase-free water.
6. Place electrode cleaner in the Agilent 2100 bioanalyzer.
7. Close the lid and leave closed for 10 seconds.
8. Open the lid and remove the electrode cleaner.
9. Wait another 10 seconds for the water on the electrodes to evaporate.
10. Remove RNaseZAP and RNase-free water out of the electrode cleaner at the end of the day.

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 well when dispensing liquids. Placing the pipette at the edge of the well may lead to bubbles and poor results.
- Keep all reagents and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to warm up to room temperature for 30 min 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 exactly 1 minute at the indicated setting (2400 rpm).
- Use a new syringe and electrode cleaners with each new LabChip, Kit.
- Use RNase-free tips and tubes and always wear gloves when handling RNA.
- Heat denature samples and RNA ladder at 70 °C for 2 min.

RNA 6000 Nano Analytical Specifications

Specification	Total RNA Assay	mRNA Assay
Quantitative range	25–500 ng/µl	25–250 ng/µl
Qualitative range	5–500 ng/µl	25–250 ng/µl
Maximum sample buffer strength	10 mM Tris-0.1 mM EDTA	10 mM Tris-0.1 mM EDTA
Reproducibility of quantitation	10 % CV	10 % CV

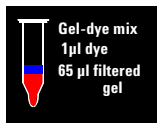
RNA 6000 Nano Assay Protocol - Edition November 2003

Preparing the Gel

1 Put 550 µl of RNA 6000 Nano gel matrix (red ●) into a spin filter.
2 Centrifuge at 1500 g ± 20 % for 10 minutes.
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 Nano dye concentrate (blue ●) to equilibrate to room temperature for 30 min.
2 Vortex RNA 6000 Nano 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 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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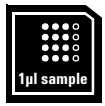
Loading the RNA 6000 Nano Marker

1 Pipette 5 µl of RNA 6000 Nano Marker (green ●) in well marked and in all 12 sample wells.
Add 6 µl of RNA 6000 Nano Marker (green ●) to each unused well.

Loading the Ladder and Samples

1 Pipette 1 µl of RNA 6000 ladder in well marked .
2 Pipette 1 µl of sample in each of the 12 sample wells. Pipette 1 µl of RNA 6000 Nano Marker (green ●) in each unused sample well.
3 Put the chip in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
4 Run the chip in the Agilent 2100 bioanalyzer 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 potential mutagen and used wih 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