

Agilent RNA 6000 Nano Kit Quick Start Guide

Agilent RNA 6000 Nano Kit (reorder number 5067-1511)

RNA Chips

25 RNA Nano Chips
2 Electrode Cleaners

Reagents (reorder number 5067-1512) & Supplies

- (yellow) Agilent RNA 6000 Ladder (reorder number 5067-1529)
- (blue) RNA Nano Dye Concentrate*
- (green) Agilent RNA 6000 Nano Marker (2 vials)
- (red) Agilent RNA 6000 Nano Gel Matrix (2 vials)
- 4 Spin Filters

Syringe Kit

1 Syringe

Tubes for Gel-Dye Mix

30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free) 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 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 only.

Assay Kits

Agilent RNA kits are designed for the analysis of total RNA (eukaryotic and prokaryotic) and mRNA samples. The complete RNA 6000 Nano kit guide can be found in the online help of the 2100 expert software. Other RNA kits: Agilent RNA 6000 Pico kit (reorder-no 5067-1513), Agilent Small RNA Kit (reorder-no 5067-1548).

Storage Conditions

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

Equipment Supplied with the Agilent 2100 Bioanalyzer

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



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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 (≥ 1300 g)
- Heating block or water bath for ladder/sample preparation

Sample Preparation

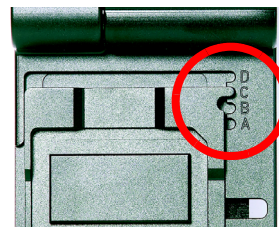
- For total RNA or mRNA analysis, the sample concentration must be in the specified range. If concentration of your particular sample is above this range, dilute with RNase-free water.

Physical Specifications		Analytical Specifications		
Type	Specification	Specification	Total RNA Assay	mRNA Assay
Analysis run time	30 minutes	Quantitative range	25–500 ng/µl	25–250 ng/µl
Number of samples	12 samples/chip	Qualitative range	5–500 ng/µl	25–250 ng/µl
Sample volume	1 µl	Quantitation accuracy	20 % CV (for ladder as sample)	20 % CV (for ladder as sample)
Kit stability	4 months (Storage temperature see individual box!)	Buffer compatibility*	100 mM Tris or 125 mM NaCl or 15 mM MgCl ₂	100 mM Tris or 125 mM NaCl or 15 mM MgCl ₂
		Reproducibility of quantitation	10 % CV	10 % CV

*) 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 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.



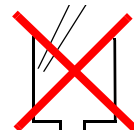
3 Adjust the syringe clip:

- 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 minutes before use. Keep 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, microfuge tubes and water.
- It is recommended to heat denature all RNA samples and RNA ladder before use (“Preparing the RNA ladder after arrival” on page 12 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), 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.



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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

- | | |
|--|--|
| <ol style="list-style-type: none"> 1 After reagent kit arrival pipette the Ladder in RNase-free vial. 2 Heat denature it for 2 min at 70 °C. 3 Immediately cool down the vial on ice. | <ol style="list-style-type: none"> 4 Prepare aliquots in RNase-free vials with the required amount for a typical daily use. 5 Store aliquots at -70°C. After initial heat denaturing, the frozen aliquots needn't be heat denatured again. 6 Before use thaw ladder aliquots on ice (avoid extensive warming) |
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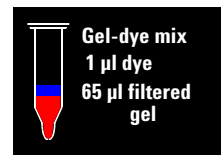
Agilent RNA 6000 Nano Assay Protocol - Edition April 2007

Preparing the Gel

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

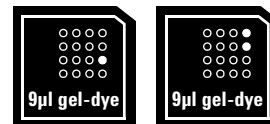
Preparing the Gel-Dye Mix

- 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 μ l of dye into a 65 μ l 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 Nano chip on the chip priming station.
- 2 Pipette 9.0 μ l 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 chip priming station and pipette 9.0 μ l of gel-dye mix in the wells marked **G**.
- 8 Discard the remaining gel-dye mix.



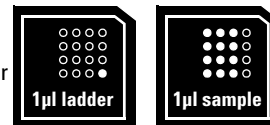
Loading the Agilent RNA 6000 Nano Marker

- 1 Pipette 5 μ l of RNA 6000 Nano marker (green ●) in all 12 sample wells and in the well marked **M**.



Loading the Ladder and Samples

- 1 Pipette 1 μ l of prepared ladder in well marked **L**.
- 2 Pipette 1 μ l of sample in each of the 12 sample wells. Pipette 1 μ l of RNA 6000 Nano Marker (green ●) in each unused sample well.
- 3 Put the chip horizontally in the adapter of the IKA vortexer and vortex for 1 min at 2400 rpm.
- 4 Run the chip in the Agilent 2100 bioanalyzer within 5 min.



Technical Support In the U.S./Canada: 1-800-227-9770 (toll free); lscs-ibs-support@agilent.com. In Europe: call your local Customer Care Center; bio_solutions@agilent.com. In Japan: 0120 477 111; yan_ccr@agilent.com. In Asia Pacific: call your local Customer Care Center; Bioanalyzer_ap@agilent.com

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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