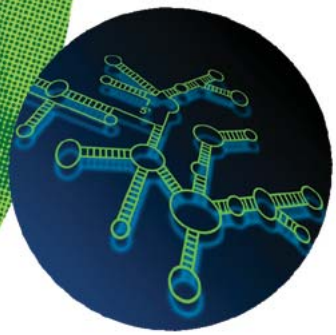
caliper LabChip®

Reagent Kit Guide
RNA 6000 Nano Assay

Edition April 2003



Agilent Technologies

Edition April 2003

Part number: G2941-90126

Contents

RNA 6000 Nano Assay Kit

Make sure that your RNA 6000 Nano LabChip® kit comes with the following items:

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

RNA 6000 Nano Chips

25 RNA Nano Chips

2 Electrode Cleaners

RNA 6000 Nano Reagents & Supplies (reorder number 5065-4475)

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

4 Spin Filters

Tubes for Gel-Dye Mix

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

Syringe Kit

1 Syringe

* RNA dye concentrate is manufactured by Molecular Probes, Inc. and licensed for research use only.

Check www.agilent.com/chem/labonachip for new details on assays.

Required Equipment for RNA 6000 Nano Assay

Supplied with the Agilent 2100 bioanalyzer

- Chip Priming Station (reorder number 5065-4401)
- IKA Vortex mixer

Additional Equipment needed for RNA 6000 Nano Assay (not supplied)

- RNA 6000 ladder for quantitation of RNA samples (Ambion, Inc. cat. no. 7152)
- RNaseZAP® recommended for electrode decontamination (Ambion, Inc. cat. no. 9780)
- RNase-free water
- Pipettes (10 µl and 1000 µl) with compatible tips (RNase-free, filter tips recommended)
- 0.5 ml and 1.5 ml microcentrifuge tubes (RNase-free)
- Microcentrifuge
- Heating block or water bath for ladder/sample denaturation
- Recommended: 16-pin bayonet electrode cartridge (reorder number 5065-4413)

Setting up the Assay Equipment and Bioanalyzer

Before beginning the chip preparation protocol, ensure that the Chip Priming Station and the bioanalyzer are set up and ready to use.

You have to

- set up the Vortex Mixer,
- replace the syringe at the Chip Priming Station with each new kit,
- adjust the base plate of the Chip Priming Station,
- adjust the syringe clip at the Chip Priming Station, and
- adjust the bioanalyzer's chip selector.
- Finally, make sure that you start the software before you load the chip.

NOTE **The RNA 6000 Nano assay is a high sensitivity assay. Please read this guide carefully and follow all instructions to guarantee satisfactory results.**

Vortex Mixer: IKA (Model MS2-S8/MS2-S9)

Setting up the Chip Priming Station

NOTE **Replace the syringe with each new Reagent Kit.**

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) Insert the new syringe into the clip.

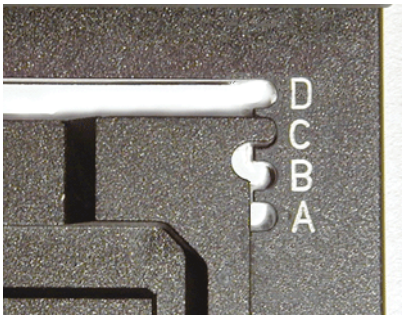
d) Slide it into the hole of the luer lock adapter and screw it tight.

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 in position C. Retighten the screw.



3 Adjust the syringe clip:

Release the lever of the clip and slide it up to adjust it to the top position.

Setting up the Bioanalyzer

Adjust the chip selector:

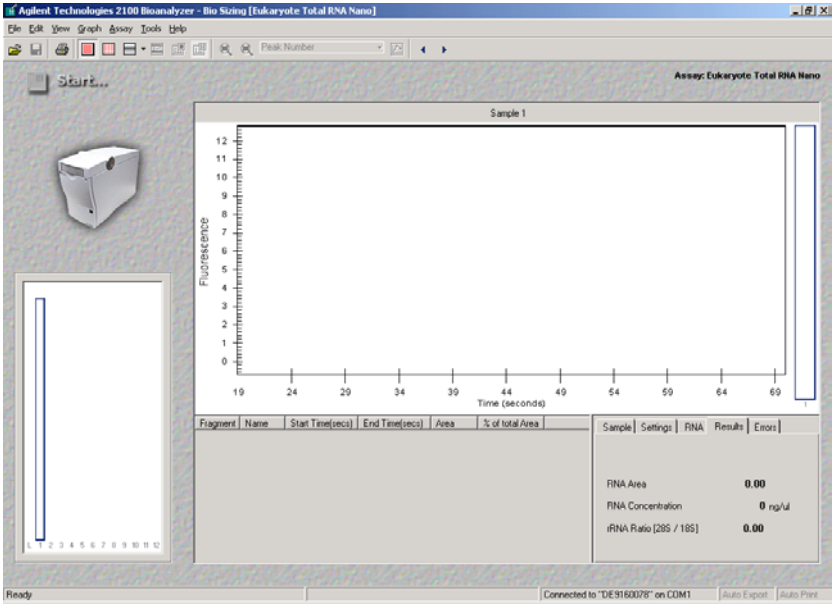
1. Open the lid of the bioanalyzer and make sure that the electrode cartridge is inserted in the instrument.
2. Remove any remaining chip.
3. Adjust the chip selector to position (1).

Starting the Agilent 2100 Bioanalyzer Software

To start the software:

1 Go to your desktop and double-click the following icon.

The main screen of the software appears. The icon in the upper left corner of the screen represents the current status as follows:



Lid closed, no chip or chip empty

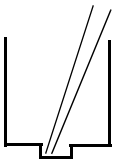
Lid open

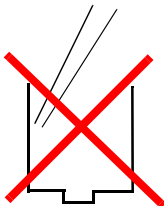
Dimmed icon: no communication

Lid closed and chip inserted, RNA or RNA demo assay selected

Essential Measurement Practices

- Handle and store all reagents according to the instructions.
- 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 (70 °C, 2 minutes).
- 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.
- Keep all reagent and reagent mixes refrigerated at 4 °C when not in use.
- Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use.
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye mix.
- Protect dye and gel-dye mix from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- 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 bubbles and poor results.





- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 minutes. Reagents might evaporate, leading to poor results.
- Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on vibrating surface.

RNA 6000 Nano Assay Protocol

After completing the initial steps in "Setting up the Assay Equipment and Bioanalyzer" on page 7, you can prepare the assay, load the chip, and run the assay, as described in the following procedures.

NOTE

If you use the RNA 6000 Nano Assay kit for the first time, you must read these detailed instructions. If you have some experience, you might want to use the RNA 6000 Nano Assay Quick Reference Guide at the end of this guide.

Decontaminating the Electrodes

NOTE Perform the following RNase decontamination procedure on a daily basis before running any assays.

1. Slowly fill one of the wells of an electrode cleaner with 350 µl RNaseZAP.
2. Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
3. Close the lid and leave it closed for about 1 minute.
4. Open the lid and remove the electrode cleaner—label the electrode cleaner and keep it for future use. You can reuse the electrode cleaner for all the chips in the kit.
5. Slowly fill one of the wells of *another* electrode cleaner with 350 µl RNase-free water.
6. Place electrode cleaner in the Agilent 2100 bioanalyzer.
7. Close the lid and leave it closed for about 10 seconds.
8. Open the lid and remove the electrode cleaner—label it and keep it for further use.
9. Wait another 10 seconds for the water on the electrodes to evaporate before closing the lid.

NOTE Remove the RNaseZAP and the RNase-free water out of the electrode cleaner at the end of the day.

Preparing the Gel

1. Allow all reagents to equilibrate to room temperature for 30 minutes before use.
2. Place 550 µl of RNA 6000 Nano gel matrix (red ●) into the top receptable of a spin filter.
3. Place the spin filter in a microcentrifuge and spin for 10 minutes at 1500 g ± 20 % (for Eppendorf microcentrifuge, this corresponds to 4000 rpm).
4. Discard the filter according to good laboratory practices. Aliquot 65 µl filtered gel into 0.5 ml RNase-free microfuge tubes that are included in the kit. Store the aliquots at 4 °C and use them within one month of preparation.

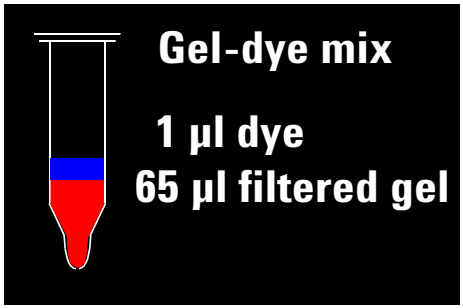
Preparing the Gel-Dye Mix

WARNING **Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.**
Kit components contain DMSO. 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.

1 Allow all reagents to equilibrate to room temperature for 30 minutes before use. Protect the dye concentrate from light while bringing it to room temperature.

2 Vortex RNA 6000 Nano dye concentrate (blue ●) for 10 seconds and spin down.

3 Add 1 µl of dye to a 65 µl aliquot of filtered gel (prepared as described in "Preparing the Gel" on page 17).



4 Cap the tube, vortex thoroughly and visually inspect proper mixing of gel and dye. Store the dye concentrate at 4°C in the dark again.

5 Spin tube for 10 minutes at room temperature at 13000 g (for Eppendorf microcentrifuge, this corresponds to 14000 rpm). Use prepared gel-dye mix within one day.

NOTE A larger volume of gel-dye mix can be prepared in multiples of the 65+1 ratio, if more than one chip will be used within one day. Always re-spin the gel-dye mix at 13000 g for 10 minutes before each use.

Loading the Gel-Dye Mix

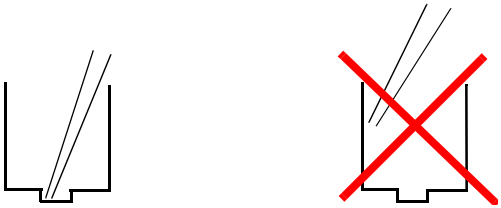
NOTE

Make sure that the Chip Priming Station base plate is in the correct position (C) before loading the gel-dye mix. Make also sure that the adjustable clip is set to the Upper position. Refer to "Setting up the Chip Priming Station" on page 9 for details.

1 Take a new RNA Nano chip out of its sealed bag.

2 Place the chip on the Chip Priming Station.

3 Pipette 9.0 µl of the gel-dye mix at the bottom of the well marked G and dispense the gel-dye mix. When pipetting the gel-dye mix make sure not to draw up particles that may sit at the bottom of the gel-dye mix vial. Insert the tip of the pipette to the bottom of the chip well when dispensing. This prevents a large air bubble forming under the gel-dye mix. Placing the pipette at the edge of the well may lead to poor results.



4 Set the timer to 30 seconds. Make sure that the plunger is at 1 ml, then close the Chip Priming Station. The lock of the latch will click when the Priming Station is closed correctly.

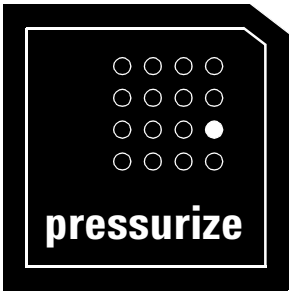
5 Press the plunger until it is held by the syringe clip.

6 Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.

7 Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.

8 Open the Chip Priming Station.

9 Pipette 9.0 µl of the gel-dye mix in each of the wells marked **G**.



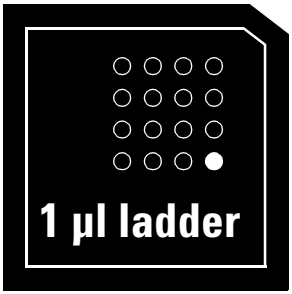
NOTE Please discard the remaining gel-dye mix.

Loading the RNA 6000 Nano Marker

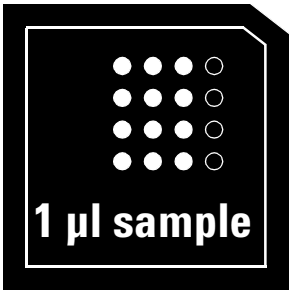
1 Pipette 5 µl of the RNA 6000 Nano Marker (green ●) into the well marked with the ladder symbol and each of the 12 sample wells.

NOTE Do not leave any wells empty or the chip will not run properly. Add 6 µl of the RNA 6000 Nano Marker (green ●) to each unused sample well.

Loading the Ladder and Samples



1. Aliquot the amount of RNA 6000 ladder (Ambion Inc., cat. no. 7152) that you use within a day into a RNase-free microcentrifuge tube and heat denature it for 2 minutes at 70°C before use.
2. Pipette 1 µl of the ladder into the well marked with the ladder symbol .
3. To minimize secondary structure, heat denature (70°C, 2 minutes) the samples before loading on the chip.



4. Pipette 1 µl of each sample into each of the 12 sample wells.
5. Place the chip in the adapter of the vortex mixer. Vortex for 1 minute at the IKA vortexer set-point (2400 rpm). If there is liquid spill at the top of the chip, carefully remove it with a tissue.
6. Refer to the next topic on how to insert the chip in the Agilent 2100 bioanalyzer. Make sure that the run is started within 5 minutes.

Inserting the Chip in the Agilent 2100 Bioanalyzer

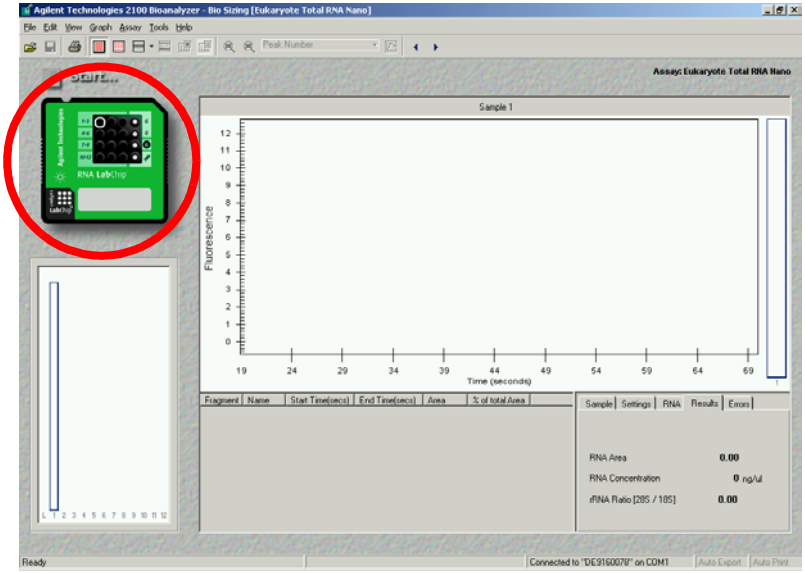
1 Open the lid of the Agilent 2100 bioanalyzer. Check that the electrode cartridge is inserted properly and the chip selector is in position (1). Refer to "Setting up the Bioanalyzer" on page 11 for details.

2 Place the chip into the receptacle. The chip fits only one way. Do not use force.

3 Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip.

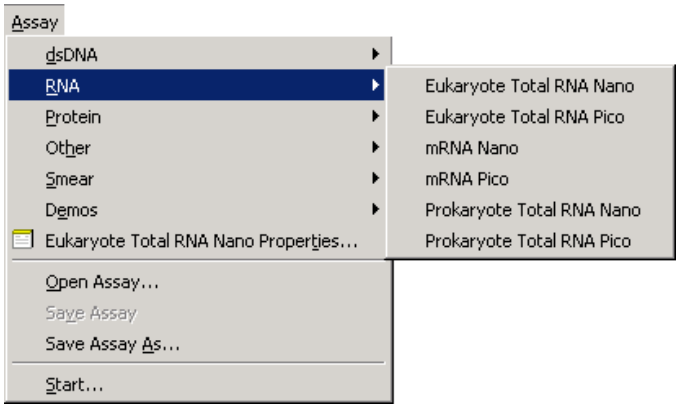
CAUTION Do not force the lid close. Otherwise electrodes may be damaged.

4 The Agilent 2100 bioanalyzer software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the left of the screen:

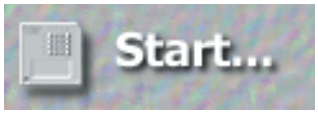


Running the RNA Assay

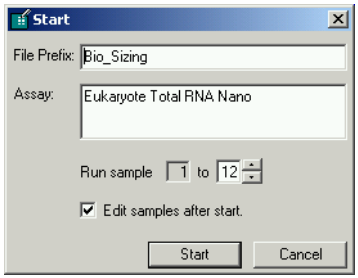
1 In the software screen select the appropriate assay from the *Assay* menu.



2 Click the *Start* button in the upper left corner of the window to open the *Start* dialog box.



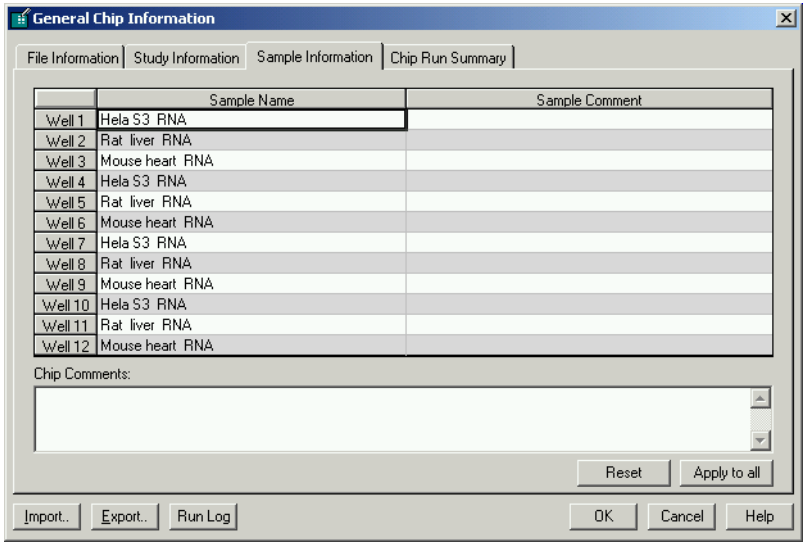
3 When the *Start* dialog box appears, the name of the loaded assay is listed as the current assay. You can enter a new prefix at this time. Data will be saved automatically to a file with a name using the prefix you have just entered. Click the *Start* button of the dialog to begin the assay.



NOTE

If the error message *Poor Chip Performance* occurs, there is not enough liquid in the wells. Prepare another chip and make sure that the chip priming has worked and to dispense all liquid from the pipette into the wells. Insert the tip of the pipette to the bottom of the well. Do not leave any wells empty.

4 If appropriate, complete the sample name table and press *OK*.



5 After the run begins, the *Start* button on the Agilent 2100 bioanalyzer software screen changes to *Stop*.

If you stop the run, all data that was collected up to the stop point will be saved.

6 When the assay is complete, remove the chip from the receptacle of the Agilent 2100 bioanalyzer and dispose of it according to good laboratory practices.

Cleaning up after an RNA Assay

When the assay is complete, *immediately* remove the used chip from the Agilent 2100 bioanalyzer and dispose of it according to good laboratory practices. Then perform the following procedure to ensure that the electrodes are clean (no residues are left over from the previous assay).

NOTE **Use a new electrode cleaner with each new kit.**

1 Slowly fill one of the wells of the electrode cleaner with 350 µl RNase-free water.

CAUTION **Never fill too much water in the electrode cleaner. This could cause liquid spill which might cause leak currents between the electrodes.**

2 Open the lid and place the electrode cleaner in the Agilent 2100 bioanalyzer.

3 Close the lid and leave it closed for about 10 seconds.

4 Open the lid and remove the electrode cleaner.

5 Wait another 10 seconds to allow the water on the electrodes to evaporate.

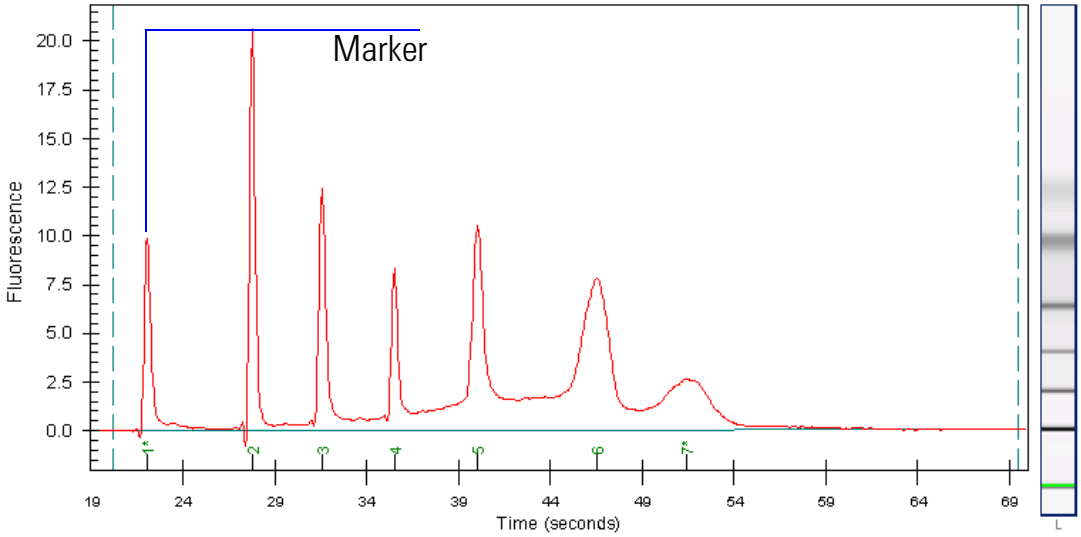
NOTE Remove the RNase-free water out of the electrode cleaner at the end of the day. **For a more thorough cleaning of the electrodes, refer to the *Maintenance and Troubleshooting Guide* .**

Checking the RNA Assay Results

You can view results for individual wells when data is acquired or after the run is finished. Click a well in the chip icon, a single well displayed on the large 12-well display, or a lane in the gel. Data regarding this well appears in a result table at the bottom of the display.

RNA Ladder Well Results

To check the results of your run, go to *View* > *Single Wells* and select the ladder well (left-most in the *Gel View* window). The electropherogram of the ladder well window should resemble the one shown here.



Major features of a successful ladder run are:

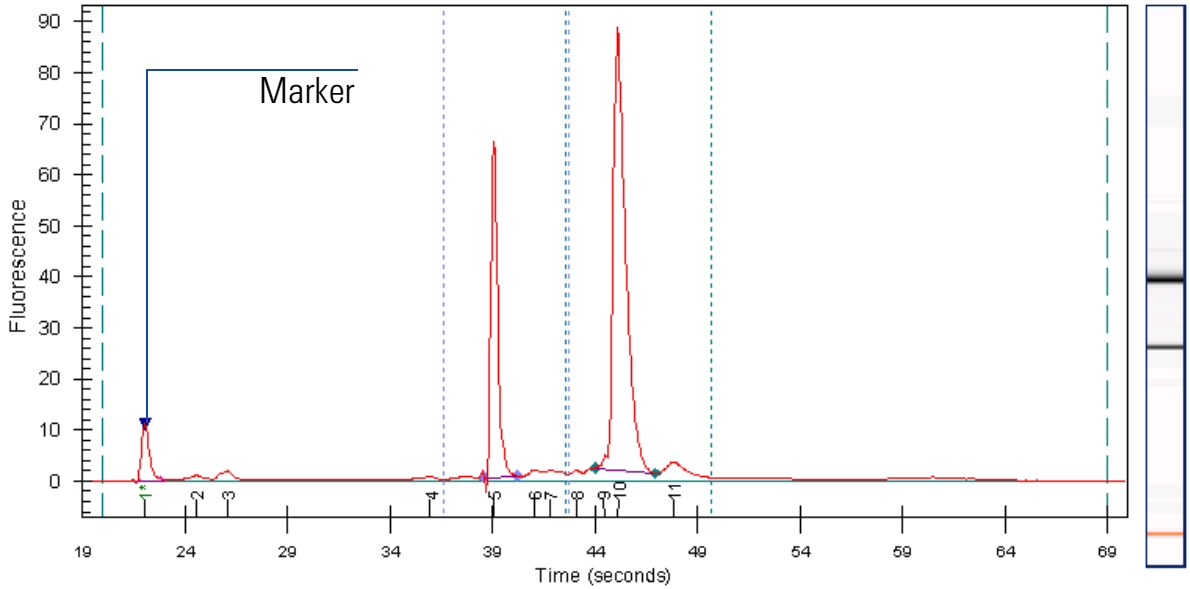
- 6 RNA peaks (s/w calls for 5 ladder peaks only)
- 1 Marker peak
- all 7 peaks are well resolved

If the electropherogram of the ladder well window does not resemble the one shown above, refer to the *Maintenance and Troubleshooting Guide* for assistance.

NOTE **The software might not detect the last peak, depending on the Peak Find settings. This will not affect the results although the separation might look different than expected.**

RNA Sample Well Results

If you are not viewing your results in single well mode, go to *View* > *Single Wells* and select one of the sample wells. The electropherogram of the sample well window for total RNA (eukaryotic) should resemble the one shown here.

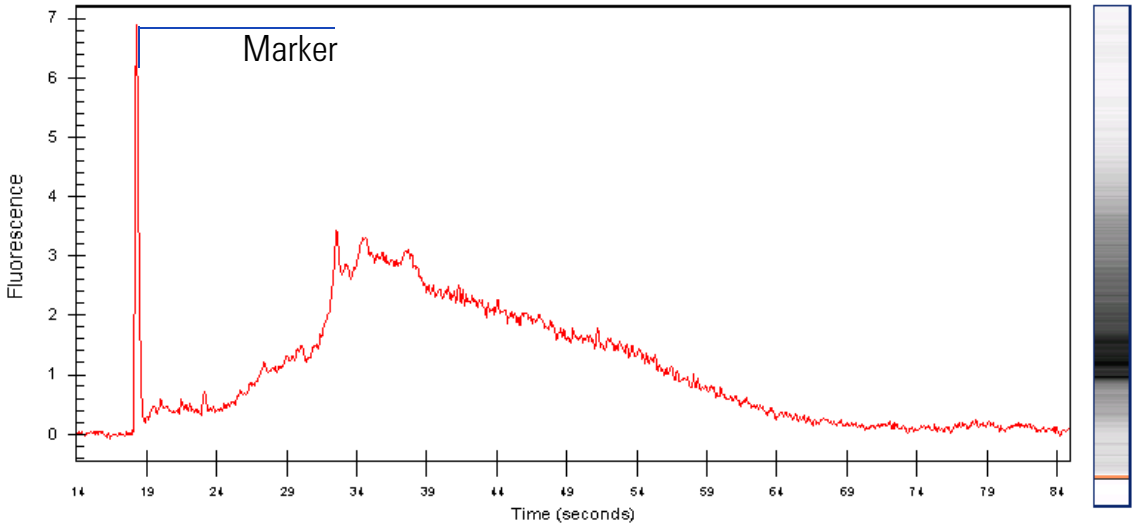


Major features for a successful total RNA run are:

- 2 ribosomal peaks (with successful sample preparation)
- 1 marker peak

NOTE **You can change the baseline by shifting the left and right dashed lines. This will alter your results.**

The electropherogram of the sample well window for mRNA should resemble the one shown here.



Major features for a successful mRNA run are:

- broad hump (with successful sample preparation)
- contamination with ribosomal RNA shown as 2 overlaid peaks (if present)
- 1 marker peak

NOTE **For information on the RNA alignment please refer to the *Users Guide* or online help.**

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RNA 6000 Nano Assay Quick Reference Guide

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

RNA 6000 Nano Chips

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

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

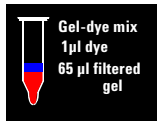
Specification	Total RNA Assay	mRNA Assay
Quantitative range	25–500 ng/µl	25–250 ng/µl
Qualitative range	5–500 ng/µl	25–250 ng/µl
Maximum sample buffer strength	10 mM Tris-0.1 mM EDTA	10 mM Tris-0.1 mM EDTA
Reproducibility of quantitation	10% CV	10% CV

RNA 6000 Nano Assay Protocol- Edition April 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, that are included in the kit. 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 Nano 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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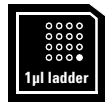
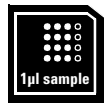
Loading the RNA 6000 Nano Marker

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 heat denatured RNA 6000 ladder in well marked ⚡.
2 Pipette 1 µl of sample in each of the 12 sample wells.
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.

Detach and keep as a reference with your instrument