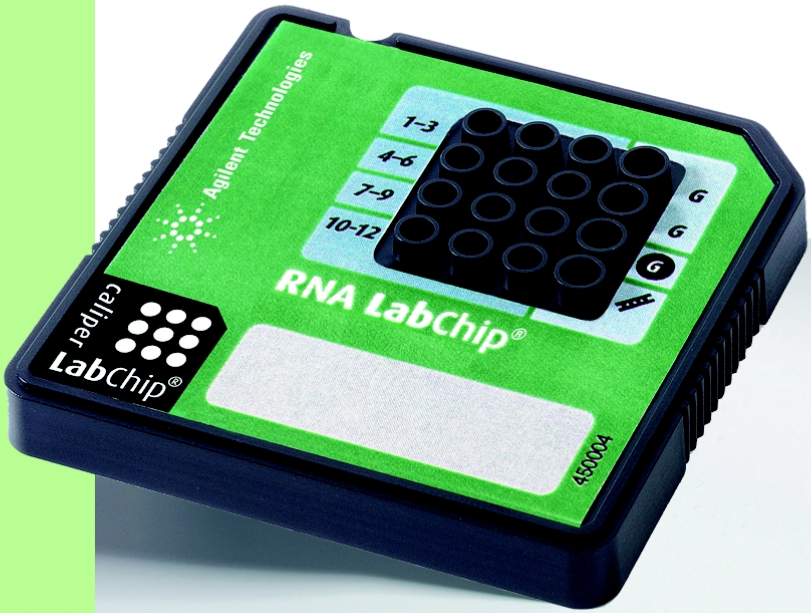
Agilent Technologies

Reagent Kit Guide
RNA 6000 Nano Assay



Welcome

This Reagent Kit Guide is intended to be used as a reference when preparing and run an RNA 6000 Nano assay with the Agilent 2100 bioanalyzer.

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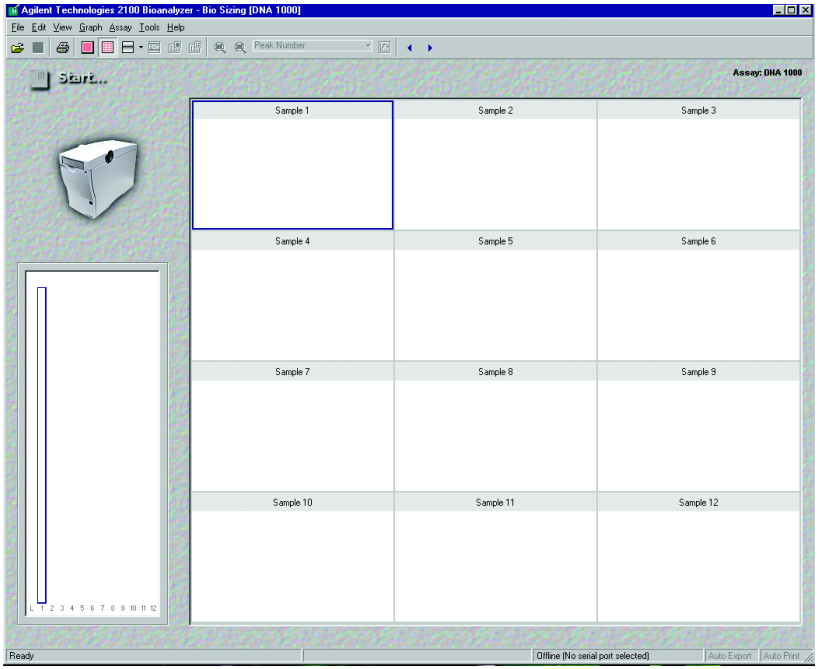
Contents

Starting the Agilent 2100 Bioanalyzer Software

To start the software, go to your desktop and double-click the icon.

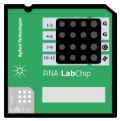
The main screen of the software appears. The Agilent 2100 bioanalyzer or chip is represented at the left side of the screen—what is shown depends on the status.



Lid closed, no chip or chip empty

Lid open

Dimmed icon: no communication



Lid closed, chip inserted, RNA or demo selected

Your First RNA Assay

If you are about to prepare and run your first RNA assay, this is the place to start. The instructions on the following pages take you through three stages.

1. Check that you have everything listed in:
   RNA 6000 Nano Assay Kit—6
   Equipment for RNA Assay (supplied with Agilent 2100 bioanalyzer)—7
   Additional Equipment for RNA Assay (not supplied)—7
2. Make sure you are familiar with the
   Essential Measurement Practices—8
3. Prepare the reagents, load the chip and run the assay as described in
   RNA Assay Protocol—9

NOTE There is a one-page quick-reference of the preparation procedures which you can find at the rear of this guide

RNA 6000 Nano Assay Kit

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

RNA Chips

25 DNA Chips

2 Electrode Cleaners

RNA 6000 Nano Reagents & Supplies

- RNA Dye Concentrate*
- RNA 6000 Nano Marker
- RNA Gel Matrix

3 Spin Filters

Syringe Kit

1 Syringe

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

Check the Agilent Lab-on-a-Chip webpage for new details on new assays:
www.agilent.com/chem/labonachip

Equipment for RNA Assay (supplied with Agilent 2100 bioanalyzer)

Chip Priming Station (reorder number 5065-4401)

Vortex mixer IKA (optional)

Additional Equipment for RNA 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)

Pipettes (10 µl and 1000 µl) with compatible tips (RNAse-free)

Microcentrifuge tubes (RNAse-free):

- 0.5 ml for buffer preparation
- 1.5 ml for gel preparation

Microcentrifuge

WARNING **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.**

Essential Measurement Practices

- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well leads to bubbles and poor results.
- Keep all reagent and reagent mixes refrigerated at 4°C when not in use.
- Allow all reagents to equilibrate to room temperature before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Prepared chips must be used within 5 minutes. Reagents might evaporate, leading to poor results.
- Vortex chips at the appropriate time of 1 minute. Inappropriate vortexing leads to poor results.
- Don't touch the Agilent 2100 bioanalyzer during an assay and never place it on vibrating surface
- Use a new syringe and cleaning chip with each new kit.
- Use RNAse free tips and tubes.
- **Aliquot the amount of ladder you use within a day into a RNase-free microcentrifuge tube and heat at 70°C for two minutes.**
- **It is recommended to heat denature all RNA samples before use (70°C, 2 min).**

RNA Assay Protocol

An assay involves the following procedures:

NOTE There is a one-page quick-reference of the preparation procedures which you can find at the rear of this guide

If you are about to load a chip for the first time, read the detailed instructions here.

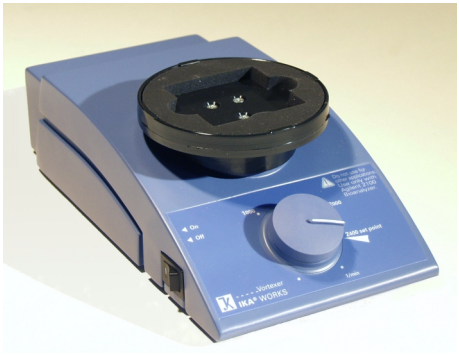
WARNING **Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. Avoid sources of dust or other contaminants. Foreign matter in reagents and samples or in the wells of the chip will interfere with assay results.**

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.

Assay Equipment Set-Up

Before following any of the chip preparation protocols, ensure that the following equipment is set up and ready to use.

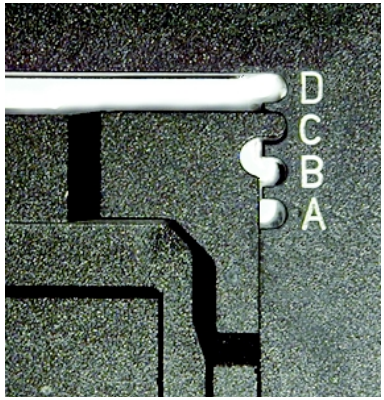
Vortex Mixer: IKA (model MS2-S9)



Chip Priming Station Set Up

Refer to the Syringe Kit and the Chip Priming Station inserts for details.

Overview



Base Plate: position C

Syringe-clip: topmost position

Decontaminating the Electrodes

Essential Practices

- *Perform the following RNAse decontamination procedure on a daily basis before running any assays.*

Procedure

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 for future use. You can reuse the electrode cleaner for all the chips in the kit. Remove the RNAseZAP at the end of the day.
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.
9. Wait another 10 seconds for the water on the electrodes to evaporate.

Preparing the Gel-Dye Mix

Essential Practices

- *Store the gel-dye mix at 4°C when not in use for more than 2 hours.*
- *Use the gel-dye mix within one week of preparation.*
- *Use the filtered gel within one month of preparation.*
- *Protect the gel-dye mix from light—the dye will degrade when exposed to light and this reduces signal intensity.*
- *Allow the gel-dye mix to equilibrate to room temperature before use.*
- *Protect the gel-dye mix from light while bringing it to room temperature.*

Procedure (sufficient for 4 chips)

1. Place 400 µl of RNA gel matrix (red ●) into the top receptable of a spin filter.
2. Place the spin filter in a microcentrifuge and spin at 1500g* ±20% for 10 minutes.
3. Place 130 µl of the filtered RNA gel matrix into an RNAse free 1.5 ml microfuge tube and add 2 µl of RNA dye concentrate (blue ●).
4. Cap the tube, vortex thoroughly and visually inspect proper mixing of gel and dye.

NOTE A larger volume of gel-dye mix can be prepared in multiples of the 130+2 ratio, if more than 4 chips will be used within 1 week.

* 4000 rpm with Eppendorf microcentifuge

Loading the Gel-Dye Mix

Essential Practices

- *Insert the tip of the pipette to the bottom of the well when dispensing. This prevents a large air bubble forming under the gel-dye mix.*

Procedure

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 page 11 of this document for details (picture).

1. Take a new RNA chip out of its sealed bag.
2. Place the chip on the Chip Priming Station.
3. Draw up 9.0 µl of the gel-dye mix with a pipette.
4. Place the tip of the pipette at the bottom of the well marked G and dispense the gel-dye mix.
5. Make sure that the plunger is at 1ml, then close the Chip Priming Station.

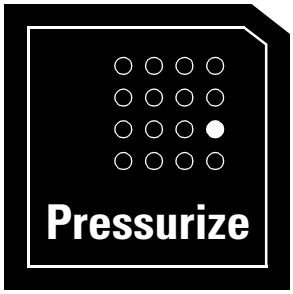
6 Press the plunger until it is held by the syringe clip.

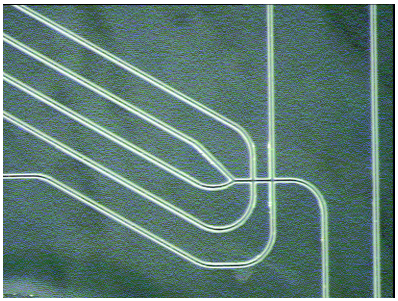
7 Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.

8 Pull back the plunger to the 1 ml position.

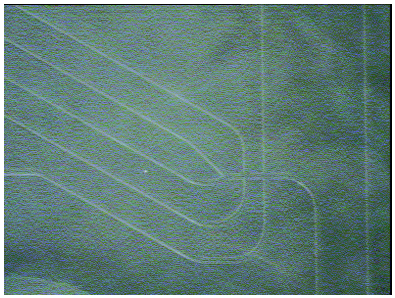
9 Open the Chip Priming Station.

10 Turn over the chip to check for air bubbles.

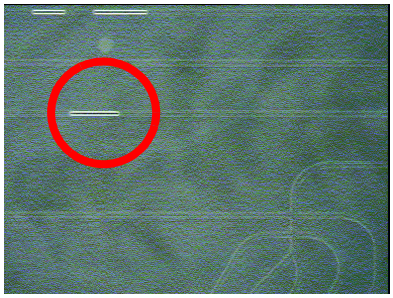




Empty chip



Loaded chip:
no air bubbles



Loaded chip:
with air bubbles

You will commonly see small bubbles in the wells after pipetting but the vortexing procedure (done after loading samples) will typically remove these bubbles. If you see bubbles in the channels of the chip, repeat steps 6 and 10. This is a fairly rare occurrence but can happen if a good seal is not formed when the chip is pressurized the first time.

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

Loading the RNA 6000 Nano Marker

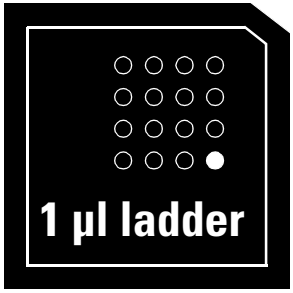
1 Draw up 5 µl of the RNA 6000 Nano Marker (green ●).

2 Place the pipette tip all the way to the bottom of the well marked with the ladder symbol. Dispense the buffer into the well.

3 Dispense 5 µl of the RNA 6000 Nano Marker (green ●) into each of the 12 sample wells.

NOTE All 12 wells must be filled with sample buffer, even when running less then 12 samples.

Loading the Ladder



1 Draw up 1 µl of the RNA 6000 ladder into a pipette.

2 Place the pipette tip all the way to the bottom of the well marked with the ladder symbol. Dispense the ladder into the well.

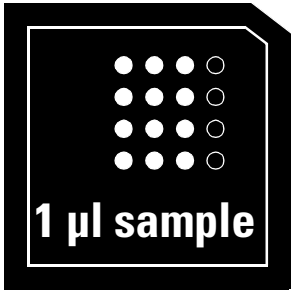
Loading the Samples

Essential Practices

- *Do not leave any wells empty or the chip will not run properly. Add an additional 1 µl of RNA 6000 Nano Marker* (green ●) *to the 5 µl of sample buffer in the unused sample well(s).*

Procedure

1. Pipette 1 µl of each sample into each of the 12 sample wells.
2. Place the chip in the adapter of the vortex mixer. Vortex for 1 minute at the IKA vortexer set-point.
3. Place the chip in the Agilent 2100 bioanalyzer and start the run within five minutes.



Inserting a Chip in the Agilent 2100 Bioanalyzer

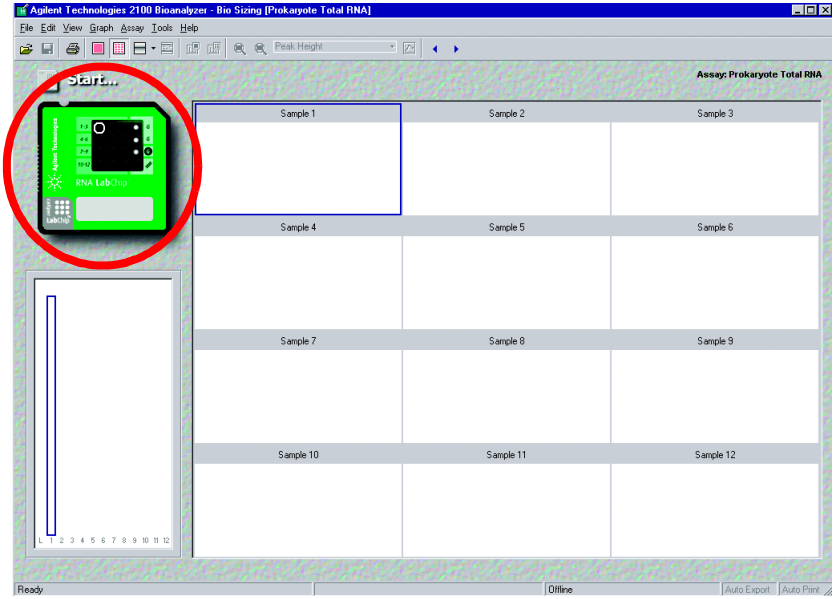
1 Open the lid of the Agilent 2100 bioanalyzer.

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

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

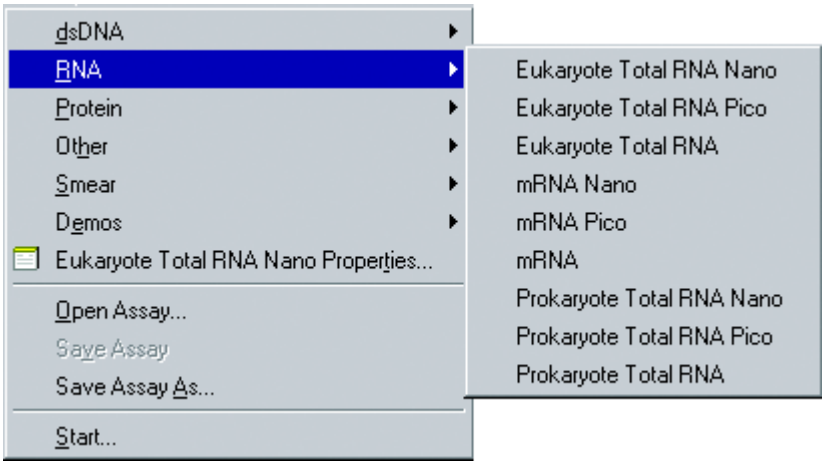
WARNING **Do not force the lid closed or electrodes may be damaged. If the lid will not shut completely, check to be sure the chip and cartridge are inserted properly and then try to close the lid again.**

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:



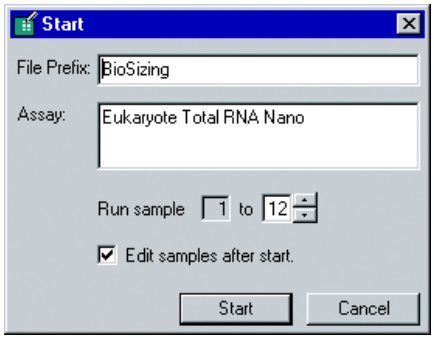
Running the RNA Assay

1 Select the appropriate assay from the Assay menu.

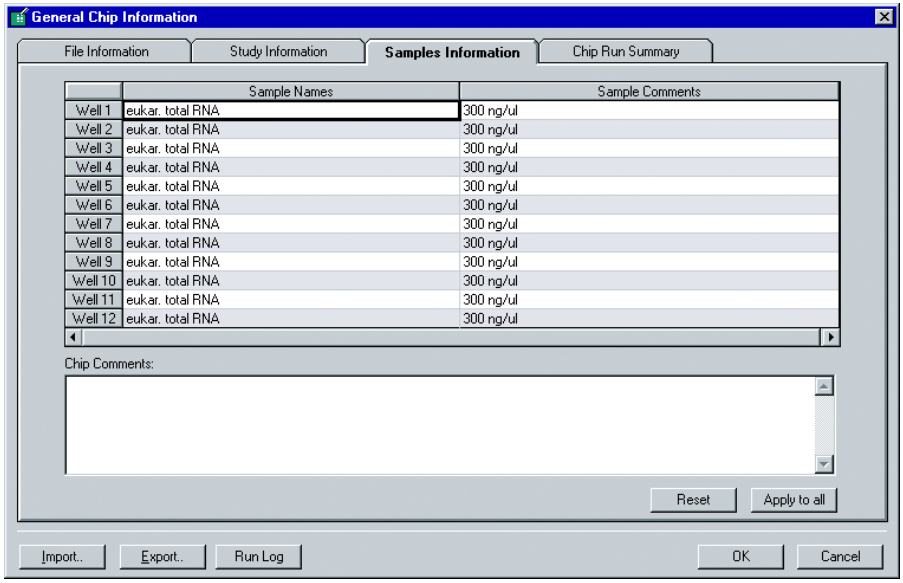


2 Position the mouse cursor over the word Start... above the icon of the chip (the word becomes active instead of dimmed). Click the Start button to open the Start dialog box.

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



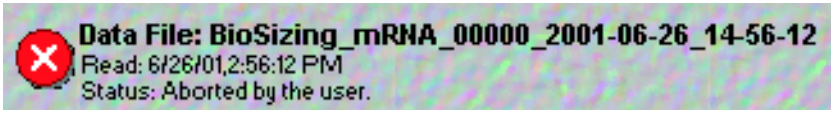
4 If appropriate, complete the sample name table and press OK.



NOTE If the error message *Voltages out of range* occurs, there is not enough liquid in the wells. Prepare another chip and make sure to dispense all the liquid from the pipette into the wells.

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

If you should need to end the run for any reason, click the Stop button. A dialog box will appear, asking if you're sure you want to end the run in progress. Clicking Yes will stop the run. The file information at the top of the screen will show a red error circle and a third line of file information will show Status: Aborted by the user.



Information collected up to the stop point is saved.

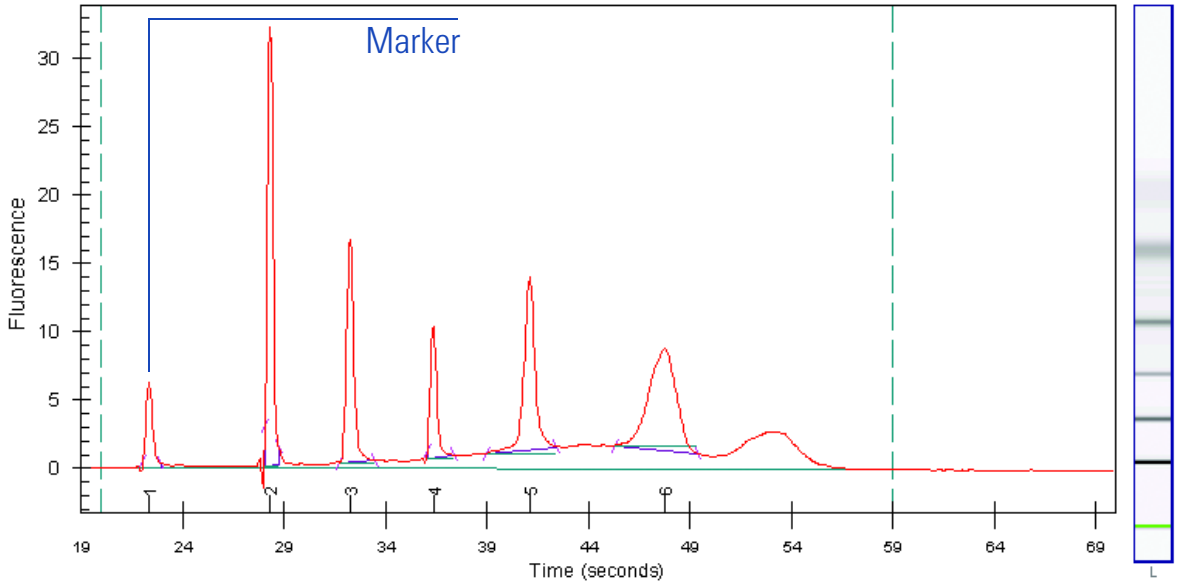
6 To view results for individual wells as data is acquired or after the run is finished, click a well in the chip, a single well displayed on the large12-well display, or a lane in the gel. Data regarding that well appears in a result table at the bottom of the display.

7 When the assay is complete, remove the chip from the receptacle of the Agilent 2100 bioanalyzer and dispose of it according to the guidelines established by your laboratory safety officer.

Checking Your RNA Results

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:



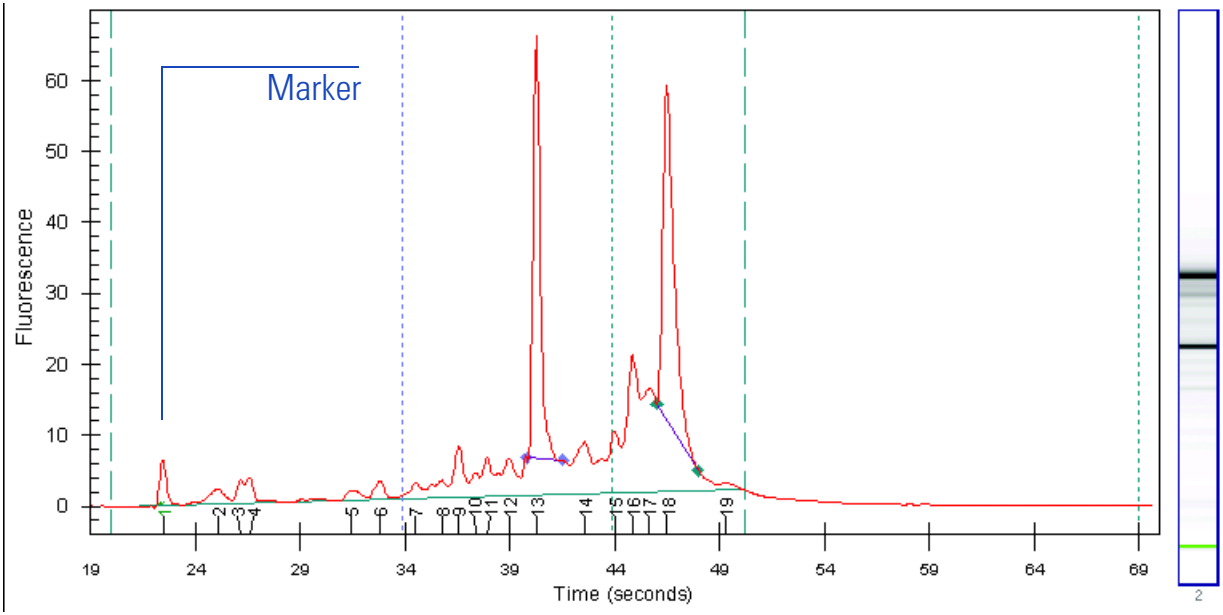
Major features of a successful ladder run are:

- 6 RNA peaks
- 1 Marker peak
- all 7 peaks are well resolved

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:

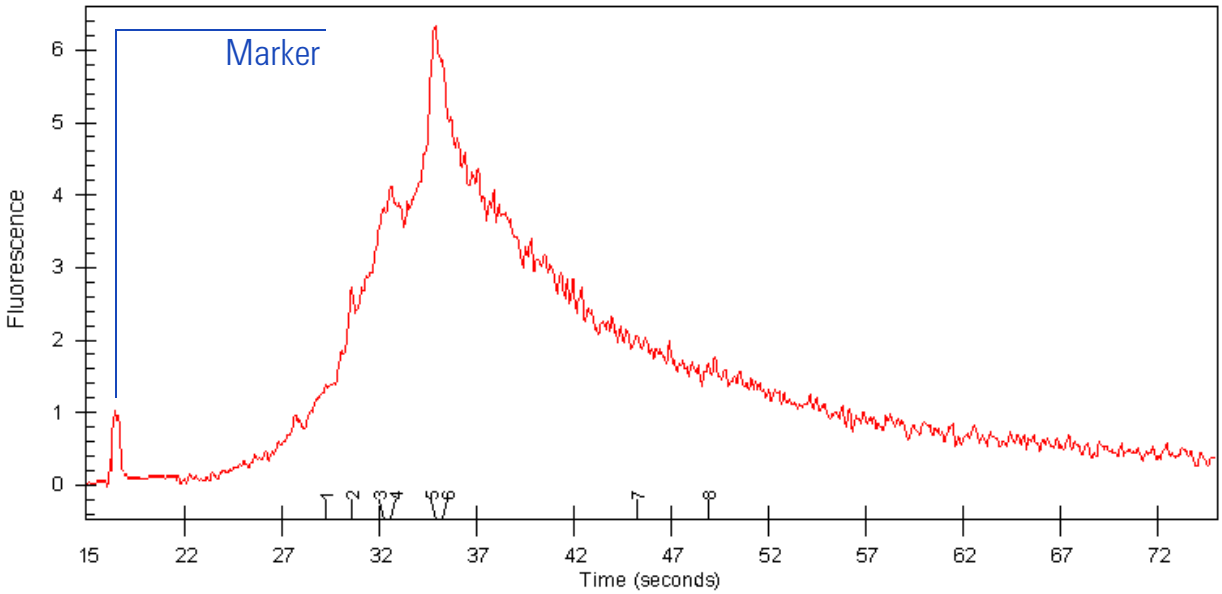


Major features for a successful total RNA run are:

- 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 online help.

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 the guidelines established by your laboratory safety officer. Then perform the following procedure to ensure that the electrodes are clean, i.e. no residues are left over from the previous assay.

Essential Practices

- *Empty and refill the electrode cleaner at regular intervals, for example, every five assays.*
- *The electrode cleaner can be used for 25 assays.*
- *Use a new cleaning chip with each new kit.*

Procedure

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

NOTE Never fill too much water in the electrode cleaner. This could cause liquid spill or contamination of the electrodes.

2 Open the lid and place 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 for the water on the electrodes to evaporate.

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Reorder number G2941-90125

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Agilent Technologies

RNA 6000 Nano Assay Quick Reference Guide

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

RNA Chips	RNA 6000 Reagents & Supplies
25 RNA Chips	RNA 6000 Dye Concentrate
2 Electrode Cleaners	RNA 6000 Nano Marker
Syringe Kit	RNA Gel Matrix
1 Syringe	3 Spin Filters

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

The RNA 6000 ladder (cat. no. 7152) must be purchased from Ambion, Inc. RNAseZAP® (cat. no. 9780) is recommended for electrode decontamination and is also available from Ambion, Inc.
RNAseZAP® is a registered trademark of Ambion, Inc.

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 sieves nucleic acid fragments by size as they are driven through it by means of electrophoresis. 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

- Pipettes (10 µl and 1000 µl) with compatible tips
- Microcentrifuge tubes
  0.5 ml for sample preparation
  1.5 ml for buffer preparation
- Microcentrifuge
- Vortex mixer from IKA

Sample Preparation

For determination of RNA concentration, total RNA in sample must be betwe
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.

Technical Support

In the U.S. call	800-227-9770
In Europe call	(+49) 7243 602 543

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

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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 before use.
- Protect dye and dye mixtures 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.
- Use a new syringe and cleaning chip with each new LabChip® Kit.

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-EDTA	10 mM Tris-EDTA
Reproducibility of quantitation	10% CV	10% CV

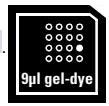
RNA 6000 Nano Assay Protocol

Preparing the Gel-Dye Mix

1 Put 400 µl of RNA gel matrix (red ●) into a spin filter.
2 Centrifuge at 1500 g ±20% for 10 min. Use filtered gel within 4 weeks.
3 Mix 130 µl of filtered RNA gel matrix with 2 µl of RNA dye concentrate (blue ●) in a RNAse free 1.5-ml microcentrifuge tube.
4 Vortex solution well. Protect solution from light. Store at 4 °C. Use within 1 week.

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 Open the chip priming station and check chip for air bubbles.
7 Pipette 9.0 µl of gel-dye mix in the wells marked G.

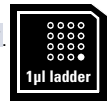


Loading the RNA 6000 Nano Marker

1 Pipette 5 µl of RNA 6000 Nano Marker (green ●) in well marked ◈.
2 Pipette 5 µl of RNA 6000 Nano Marker (green ●) in all 12 sample wells.

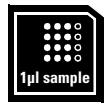
Loading the Ladder

1 Pipette 1 µl of RNA 6000 ladder in well marked ◈.



Loading the Samples

1 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.
2 Put the chip in the adapter and vortex for 1 min. at the set-point of the IKA vortexer.
3 Run the chip in the Agilent 2100 within 5 min.



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WARNING — Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples. 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. Refer to dye/DMSO warning on reverse side.

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Detach and keep as a reference by your instrument