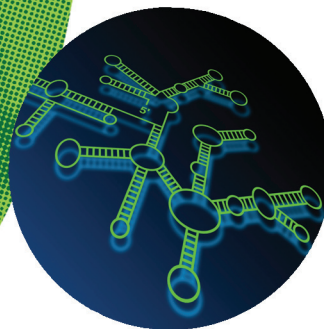




# Reagent Kit Guide **RNA 6000 Pico Assay**

Edition November 2003



**Agilent Technologies**

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<b>RNA 6000 Pico Assay Quick Reference Guide</b>	

# RNA 6000 Pico Assay Kit

Make sure that your RNA 6000 Pico LabChip<sup>®</sup> kit comes with the following items:

## **RNA 6000 Pico LabChip<sup>®</sup> Kit (reorder number 5065-4473)**

### **RNA 6000 Pico Chips**

25 RNA Pico Chips

3 Electrode Cleaners

### **RNA 6000 Pico Reagents & Supplies (reorder number 5065-4472)**

- RNA 6000 Pico Dye Concentrate<sup>\*</sup>
- RNA 6000 Pico Marker (4 vials)
- RNA 6000 Pico Conditioning Solution
- RNA 6000 Pico 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

<sup>\*</sup> RNA dye concentrate is manufactured by Molecular Probes, Inc. and licensed for research use only.

Check [www.agilent.com/chem/labonachip](http://www.agilent.com/chem/labonachip) for new details on assays.

# Required Equipment for RNA 6000 Pico Assay

## Supplied with Agilent 2100 bioanalyzer

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

## Additional Equipment needed for RNA 6000 Pico Assay (not supplied)

- RNA 6000 ladder (Ambion, Inc. cat. no. 7152)
- RNaseZAP<sup>®</sup> recommended for electrode decontamination (Ambion, Inc. cat. no. 9780)
- RNase-free water
- Pipettes (10  $\mu$ l and 1000  $\mu$ l) 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 preparation
- Mandatory: 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

- 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
- adjust the bioanalyzer's chip selector
- set up the Vortex Mixer
- Finally, make sure that you start the software before you load the chip

---

## NOTE

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

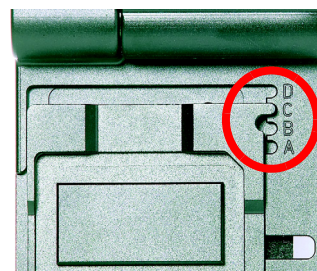
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# Setting up the Chip Priming Station

## 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 the topmost 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. If not, open the latch, remove the pressure cartridge and insert the electrode cartridge.
- 2 Remove any remaining chip and adjust the chip selector to position (1).



## Setting up the Vortex Mixer

IKA - model MS2-S8/MS2-S9

To set up the vortexer adjust the speed knob to the indicated setting (2400rpm).



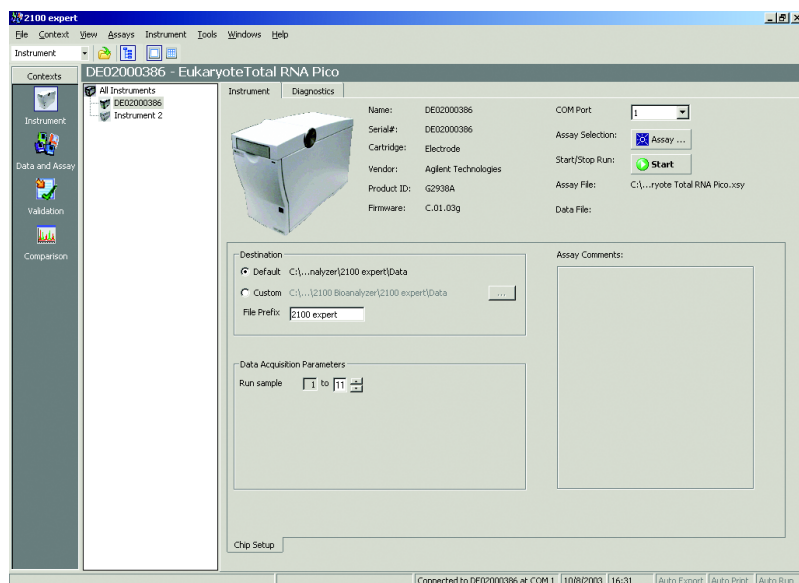
# Starting the 2100 Expert Software

To start the software:

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



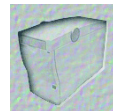
The screen of the software appears in the *Instrument* context. The icon in the upper left part of the screen represents the current instrument-PC communication status:



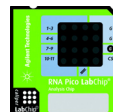
Lid closed, no chip or chip empty



Lid open



Dimmed icon: no communication



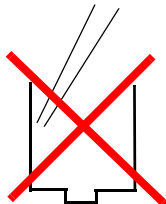
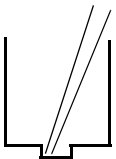
Lid closed and chip inserted, RNA Pico or demo assay selected

- 2 If more than one instrument is connected to your PC, select the instrument you want to use in the tree view.



# 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 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 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 Pico 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.

## Cleaning the Electrodes Before Running Assays

To avoid decomposition of your RNA sample, follow this electrode decontamination procedure on a daily basis before running any RNA Pico assays.

---

### NOTE

To prevent decontamination problems, 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.

---

- 1 Slowly fill one of the wells of an electrode cleaner with 350  $\mu$ l RNase free water.
- 2 Open the lid and place electrode cleaner in the Agilent 2100 bioanalyzer.
- 3 Close the lid and leave it closed for 5 minutes.
- 4 Open the lid and remove the electrode cleaner—label the electrode cleaner and keep it for future use.
- 5 Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.

## Preparing the Diluted Ladder

---

### NOTE

Always use RNase-free microfuge tubes, pipette tips and water.

---

- 1 Place 5  $\mu$ l of RNA 6000 ladder in a 1.5 ml microfuge tube and heat at 70 °C for 2 minutes, spin down the tube and keep the ladder on ice.
  - 2 Add 745  $\mu$ l of RNase-free water.
  - 3 Shortly vortex and spin down the tube.
  - 4 Store diluted RNA ladder in 10  $\mu$ l aliquots (or other volume as appropriate) at -80 °C.
- 

### NOTE

Do not heat the diluted ladder. Use defrosted diluted ladder within one day.

---



## Preparing the Gel

- 1 Allow all reagents to equilibrate to room temperature for 30 minutes before use.
- 2 Place 550  $\mu\text{l}$  of RNA 6000 Pico 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  $1500 \text{ g} \pm 20 \%$  (for Eppendorf microcentrifuge, this corresponds to 4000 rpm).
- 4 Aliquot 65  $\mu\text{l}$  filtered gel into 0.5 ml RNase-free microfuge tubes that are included in the kit. Store the aliquots at  $4 \text{ }^{\circ}\text{C}$  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 Pico dye concentrate (blue ●) for 10 seconds and spin down.
- 3 Add 1  $\mu\text{l}$  of RNA 6000 Pico dye concentrate (blue ●) to a 65  $\mu\text{l}$  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).



---

## 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.

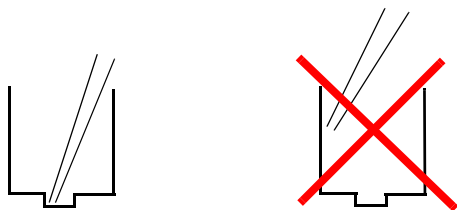
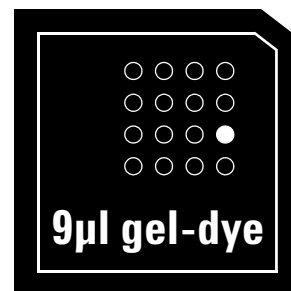
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## Loading the Gel-Dye Mix

### NOTE

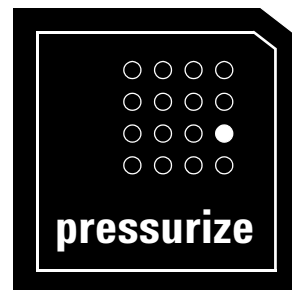
Make sure that the Chip Priming Station base plate is in the correct position (C) before loading the gel-dye mix. Make also sure that the adjustable clip is set to the Upper position. For details, please refer to “[Setting up the Chip Priming Station](#)” on page 8.

- 1 Take a new RNA Pico chip out of its sealed bag.
- 2 Place the chip on the Chip Priming Station.
- 3 Pipette 9.0  $\mu$ l 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  $\mu$ l of the gel-dye mix in each of the wells marked **G**.



---

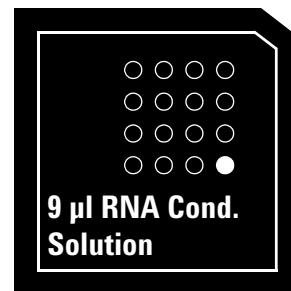
#### NOTE


Please discard the remaining gel-dye mix.

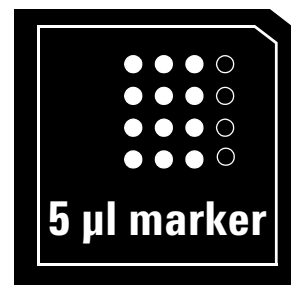
---

## Loading the RNA 6000 Pico Conditioning Solution and Marker

- 1 Pipette 9  $\mu$ l of the RNA 6000 Pico Conditioning Solution (yellow ●) into the well marked CS.



- 2 Pipette 5  $\mu$ l of the RNA 6000 Pico marker (green ●) into the well marked with a ladder symbol  and each of the 11 sample wells.



---

### NOTE


Do not leave any wells empty or the chip will not run properly. Add 6  $\mu$ l of the RNA 6000 Pico Marker (green ●) to each unused sample well.

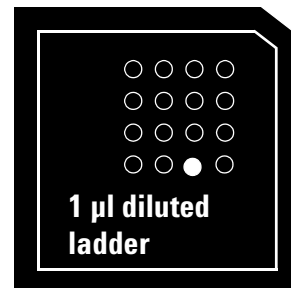
---

## Loading the Diluted Ladder and Samples

### NOTE

Always use RNase-free microfuge tubes, pipette tips and water.

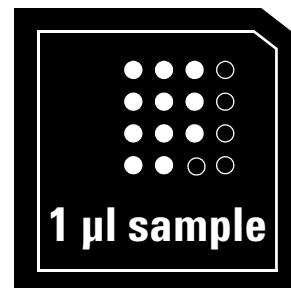
- 1 Pipette 1  $\mu$ l of the diluted RNA 6000 ladder (prepared as described in “[Preparing the Diluted Ladder](#)” on page 16) into the well marked with the ladder symbol .



### NOTE

Do not heat the diluted ladder. Use defrosted diluted ladder within one day.

- 2 To minimize secondary structure, you may heat denature (70 °C, 2 minutes) the samples before loading on the chip.
- 3 Pipette 1  $\mu$ l of each sample into each of the 11 sample wells.
- 4 Place the chip in the adapter of the vortex mixer. Vortex for 1 minute at the IKA vortexer set-point. If there is liquid spill at the top of the chip, carefully remove it with a tissue.
- 5 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.



---

## NOTE

Depending on the RNA isolation protocol, varying results can be expected. Known dependencies include: salt content, cell fixation method and tissue stain. Try to dissolve your sample in deionized water. In cases where genomic DNA might be present, include a DNase treatment in the preparation protocol.

---



## Inserting a 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 9 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 located in the cartridge fit into the wells of the chip.

---

### CAUTION

Do not force the lid closed or electrodes may be damaged.

---

- 4 The 2100 expert software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the top left of the instrument context.

2100 expert

File Context View Assays Instrument Tools Windows Help

Instrument

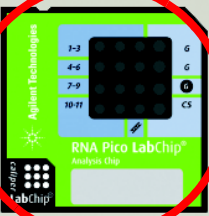
Contexts

- Instrument
- Data and Assay
- Validation
- Comparison

DE13701065 - EukaryoteTotal RNA Pico

All Instruments  
DE13701065  
Instrument 2

Instrument Diagnostics



Name: DE13701065  
Serial#: DE13701065  
Cartridge: Electrode  
Vendor: AGILENT Technologies  
Product ID: G2938B  
Firmware File: C.01.03F

COM Port: 1  
Assay Selection: Assay ...  
Start/Stop Run: Start  
Assay File: C:\...ryote Total RNA Pico.xsy  
Data File:

Destination  
 Default C:\...nalyzer\2100 expert\Data  
 Custom C:\...2100 Bioanalyzer\2100 expert\Data ...  
File Prefix: 2100 expert

Data Acquisition Parameters  
Run sample: 1 to 11

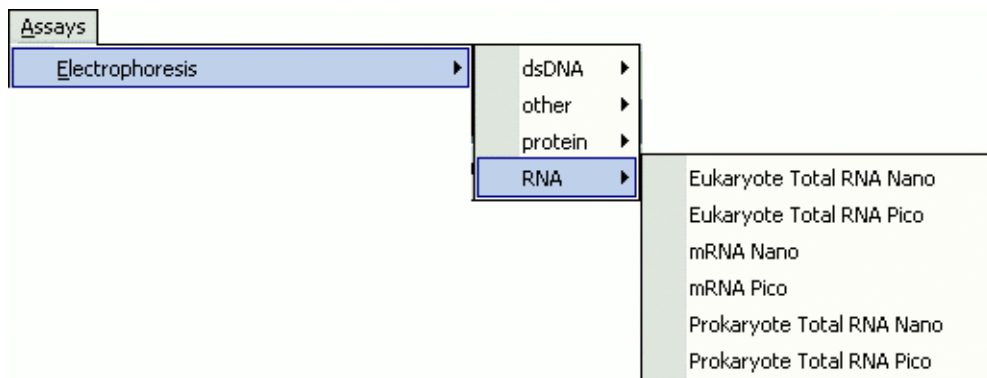
Assay Comments:

Chip Setup

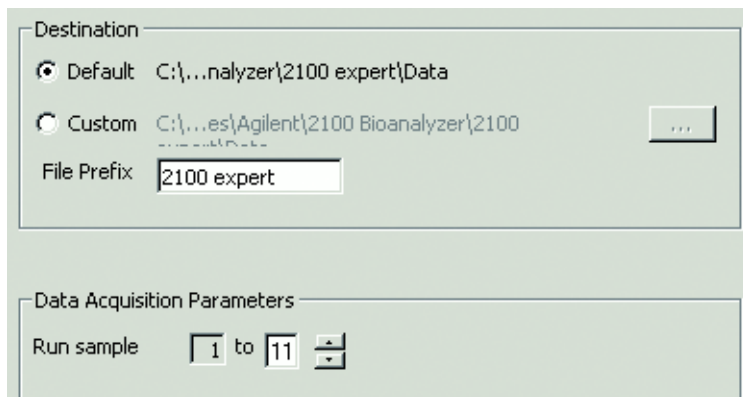
Connected to DE13701065 at COM 1 10/2/2003 15:45 Auto Export Auto Print Auto Run

# Starting the Chip Run

- 1 In the *Instrument* context, select the appropriate assay from the *Assay* menu.



- 2 Accept the current *File Prefix* or modify it. Data will be saved automatically to a file with a name using the prefix you have just entered. At this time you can also customize the file storage location and the number of samples that will be analyzed.



- 3 Click the *Start* button in the upper right of the window to start the chip run. The incoming raw signals are displayed in the *Instrument* context.



- 4 To enter sample information like sample names and comments, select the *Data File* link that is highlighted in blue or go to the *Data and Assay* context and select the *Chip Summary* tab. Complete the sample table and press *Apply*.

	Sample Name	Sample Comment	Status	Observation	Result Flagging
▶	Sample 1				
2	Sample 2				
3	Sample 3				
4	Sample 4				
5	Sample 5				
6	Sample 6				
7	Sample 7				
8	Sample 8				
9	Sample 9				
10	Sample 10				
11	Sample 11				

Chip Lot #  Reagent Kit Lot #

Chip Comments :

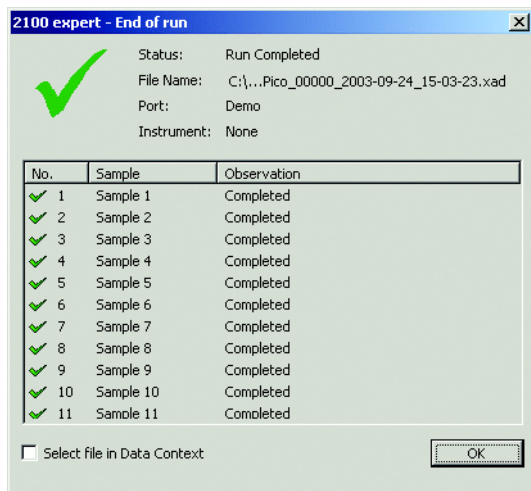
Sample Information    Study Information    Instrument Information

Import...    Export...    Apply    Cancel

- 5 To review the raw signal trace, return to the *Instrument* context.



6 When the assay is complete, the *End of Run* message appears.



7 Remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practises.

## Cleaning up after the RNA Pico Chip Run

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).

- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ l of fresh 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 electrode cleaner in the Agilent 2100 bioanalyzer.
- 3 Close the lid and leave it closed for about 30 seconds.
- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 30 seconds for the water on the electrodes to evaporate before closing the lid.

---

### NOTE

Replace the water in the cleaning chip after each use. Use a new cleaning chip after 12-13 electrode cleaning procedures and with new kit.

---

## Decontaminating the Electrodes

When you suspect the bayonet electrodes to be contaminated with RNase, perform the following decontamination procedure:

- 1 Remove the bayonet electrodes from the cartridge.
- 2 Spray RNase Zap onto electrodes and soak for 1 minute.
- 3 Gently brush the electrodes.
- 4 Rinse electrodes thoroughly with RNase free water.
- 5 Dry the electrodes by using oil-free compressed air.
- 6 Reassemble the electrode cartridge and put it back into the instrument.
- 7 To verify that the electrodes are completely dry, perform the Short Circuit Test, that you can find under the *Diagnostics* tab in the *Instrument* context.

---

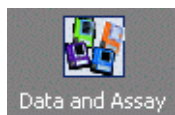
### NOTE

If the short circuit test fails, repeat previous steps.

---

# Checking Your RNA Pico Results

To review the results of a chip run, select the *Data and Assay* context.

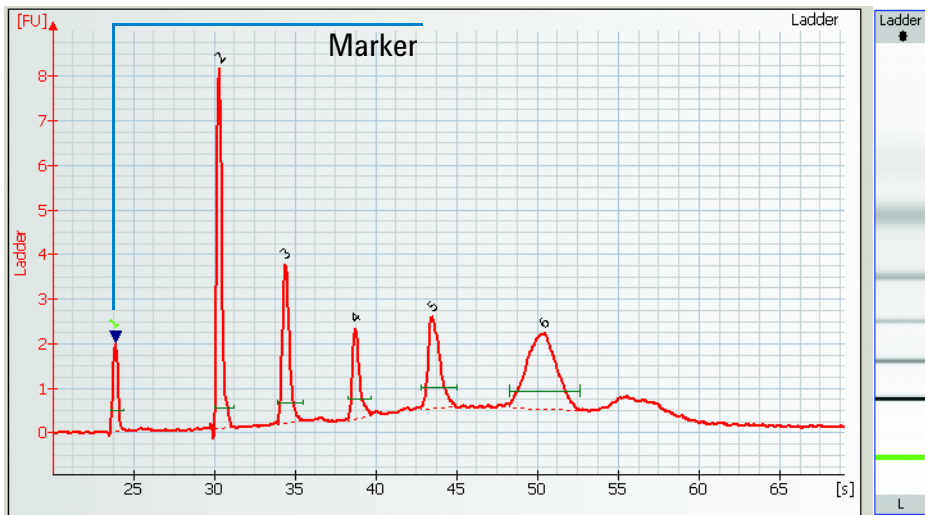


Data is displayed as electropherogram or gel-like image. To check the results of a specific sample, select the sample name in the tree view and highlight the *Results* sub-tab.

## RNA Pico Ladder Well Results

To check the results of your run, select the *Gel* or *Electropherogram* tab in the *Data and Assay* context. Select the ladder well. The electropherogram of the ladder 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 *2100 Expert Maintenance and Troubleshooting Guide* for assistance.

---

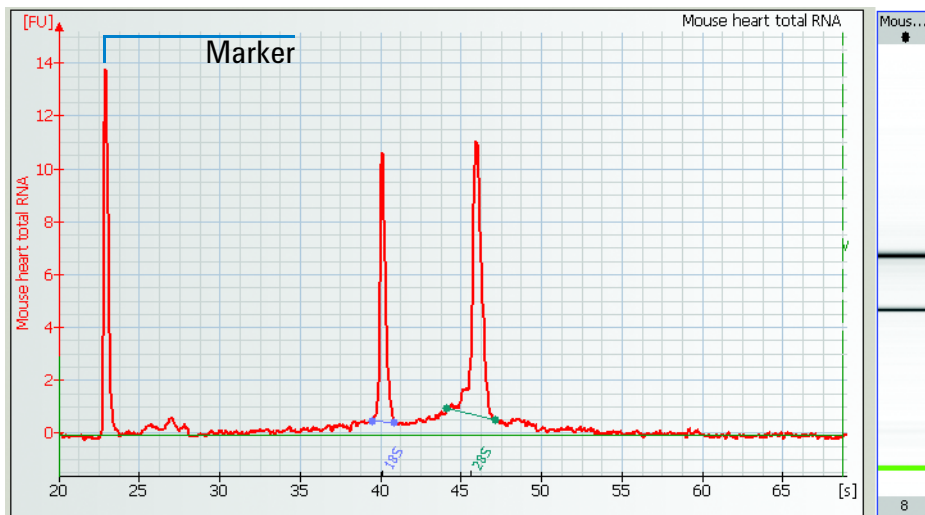
#### NOTE

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 Pico Sample Well Results

To review the results of a specific sample, select the sample name in the tree view and highlight the *Results* sub-tab. 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:

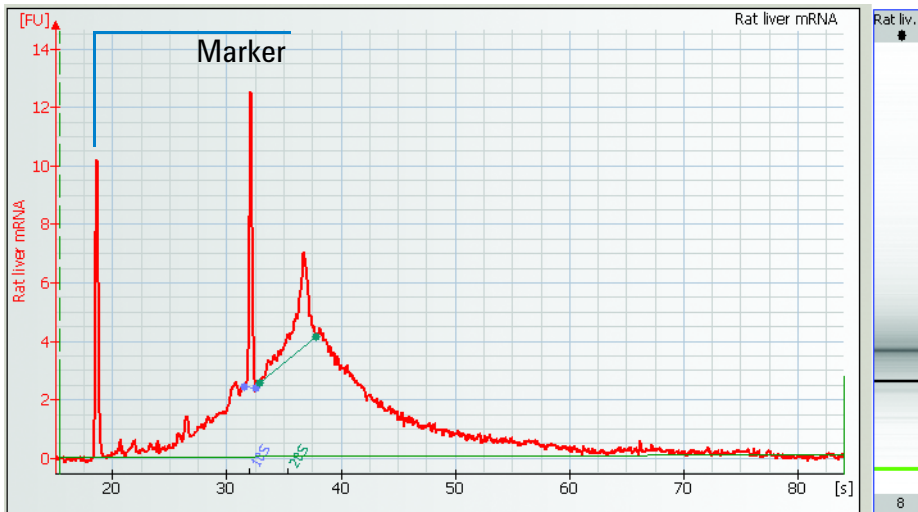
- 2 ribosomal peaks (with successful sample preparation)
- 1 marker peak

By selecting the *Results* sub-tab, values for the calculated RNA concentration and ribosomal ratio are displayed.

## NOTE

You can change the baseline by shifting the left and right dashed lines. This will alter your results. For information on the RNA alignment please refer to the *2100 Expert User's Guide* or *Online Help*.

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

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# 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
	● RNA 6000 Pico Gel Matrix (2 vials)
<b>Syringe Kit</b>	● 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 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.

### 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 procedure. 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.



### 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 **D**.



### Loading the diluted Ladder and Samples

- 1 Pipette 1 μl of diluted ladder in well marked **D**.
- 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 bioanalyser 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.



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