

# Agilent RNA 6000 Pico



**Kit Guide**



**Agilent Technologies**

# Notices

© Agilent Technologies, Inc. 2001, 2003-2008, 2013

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

## Manual Part Number

G2938-90046 Rev. B

## Edition

11/2013

Printed in Germany

Agilent Technologies  
Hewlett-Packard-Strasse 8  
76337 Waldbronn

## Research Use Only

**Not for use in Diagnostic Procedures.**

Caliper®, LabChip® and the Lab-Chip logo are registered trademarks of Caliper Technologies Corp. in the U.S. and other countries.

Adobe and Acrobat are U.S. registered trademarks of Adobe Systems Incorporated.

Microsoft® is a U.S. registered trademark of Microsoft Corporation.

### Software Revision

This guide is valid for 2100 expert B.02.04 and higher revision of the Agilent expert software, where 04 refers to minor revisions of the software that do not affect the technical accuracy of this guide.

## Warranty

**The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.**

## Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

## Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as “Commercial computer software” as defined in DFAR 252.227-7014 (June 1995), or as a “commercial item” as defined in FAR 2.101(a) or as “Restricted computer software” as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies’ standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will

receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

## Safety Notices

### CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

### WARNING

A **WARNING** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a **WARNING** notice until the indicated conditions are fully understood and met.

# Contents

<b>1</b>	<b>Agilent RNA 6000 Pico Kit</b>	<b>4</b>
<b>2</b>	<b>Required Equipment for RNA 6000 Pico Assay</b>	<b>6</b>
	Equipment Supplied with the Agilent 2100 Bioanalyzer	6
	Additional Material Required (Not Supplied)	6
<b>3</b>	<b>Setting up the Assay Equipment and Bioanalyzer</b>	<b>7</b>
	Setting up the Assay Equipment and Bioanalyzer	7
	Setting up the Chip Priming Station	8
	Setting up the Bioanalyzer	9
	Vortex Mixer	9
	Starting the 2100 Expert Software	10
<b>4</b>	<b>Essential Measurement Practices</b>	<b>11</b>
<b>5</b>	<b>Preparing the RNA Ladder after Arrival</b>	<b>13</b>
<b>6</b>	<b>Agilent RNA 6000 Pico Assay Protocol</b>	<b>14</b>
	Agilent RNA 6000 Pico Assay Protocol	14
	Cleaning the Electrodes before Running Assays	14
	Preparing the Gel	15
	Preparing the Gel-Dye Mix	16
	Loading the Gel-Dye Mix	17
	Loading the RNA 6000 Pico Conditioning Solution and Marker	18
	Loading the Diluted Ladder and Samples	19
	Inserting a Chip in the Agilent 2100 Bioanalyzer	20
	Starting the Chip Run	21
	Cleaning Electrodes after a RNA 6000 Pico Chip Run	23
	Decontaminating the Electrodes	24
<b>7</b>	<b>Checking Your Agilent RNA 6000 Pico Assay Results</b>	<b>25</b>
	RNA 6000 Pico Ladder Well Results	25
	RNA 6000 Pico Sample Well Results	26



## Kit Guide

# Agilent RNA 6000 Pico Kit

**Table 1** Agilent RNA 6000 Pico Kit (reorder number 5067-1513)

---

### **Agilent RNA 6000 Pico Chips**

25 RNA Pico Chips

3 Electrode Cleaners

*Agilent RNA 6000 Pico Reagents (reorder number 5067-1514)*

- (blue) RNA 6000 Pico Dye Concentrate<sup>1</sup>
- (green) RNA 6000 Pico Marker (4 vials)
- (white) RNA 6000 Pico Conditioning Solution
- (red) RNA 6000 Pico Gel Matrix (2 vials)
- (yellow) RNA 6000 Pico Ladder (reorder number 5067-1535) (1 vial, 10x concentrate).

4 Spin Filters

*Tubes for Gel-Dye Mix*

30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free) for gel-dye mix

*Syringe Kit*

1 Syringe

---

<sup>1</sup> "This product is provided under a license by Life Technologies Corporation to Agilent Technologies. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product only as described in accompanying product literature. The sale of this product is expressly conditioned on the buyer not using the product or its components (1) in manufacturing; (2) to provide a service, information, or data to an unaffiliated third party for payment; (3) for therapeutic, diagnostic or prophylactic purposes; (4) to resell, sell or otherwise transfer this product or its components to any third party, or use for any use other than use in the subfields of research and development, quality control, forensics, environmental analysis, biodefense or food safety testing. For information on purchasing a license to this product for purposes other than described above contact Life Technologies Corporation, Cell Analysis Business Unit, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354."



**Table 2** Physical Specifications

Type	Specification
Analysis run time	30 minutes
Number of samples	11 samples/chip
Sample volume	1 $\mu$ l
Assay kit stability	4 months (Storage temperature see individual box!)

**Table 3** Analytical Specifications

Specification	Total RNA Assay	mRNA Assay
Qualitative range	50–5000 pg/ $\mu$ l (in water) (Signal/Noise >3)	250–5000 pg/ $\mu$ l (in water) (Signal/Noise >3)
Reproducibility of quantitation	20 % CV	20 % CV
Quantitation accuracy	30 % CV (for ladder as sample)	30 % CV (for ladder as sample)
Buffer compatibility <sup>1</sup>	50 mM Tris or 50 mM NaCl	50 mM Tris or 50 mM NaCl

<sup>1</sup> Due to the high sensitivity of the assay, different ions and higher salt concentrations might influence the performance of the assay.



Kit Guide

## Required Equipment for RNA 6000 Pico Assay

### Equipment Supplied with the Agilent 2100 Bioanalyzer

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

### Additional Material Required (Not Supplied)

- RNaseZAP® 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, no filter tips, no autoclaved tips)
- 0.5 mL microcentrifuge tubes (RNase-free). Eppendorf Safe-lock PCR clean or Eppendorf DNA LoBind microcentrifuge tubes are highly recommended.
- Microcentrifuge ( $\geq 13000$  g)
- Heating block or water bath for ladder/sample preparation
- Mandatory: bayonet electrode cartridge (reorder number 5065-4413)

Check the Agilent Lab-on-a-Chip webpage for details on assays:  
[www.agilent.com/chem/labonachip](http://www.agilent.com/chem/labonachip).





## Setting up the Assay Equipment and Bioanalyzer

### 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.



## 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** 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.





## 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).



## Vortex Mixer

IKA - Model MS3

- 1 To set up the vortex mixer, adjust the speed knob to 2400 rpm.



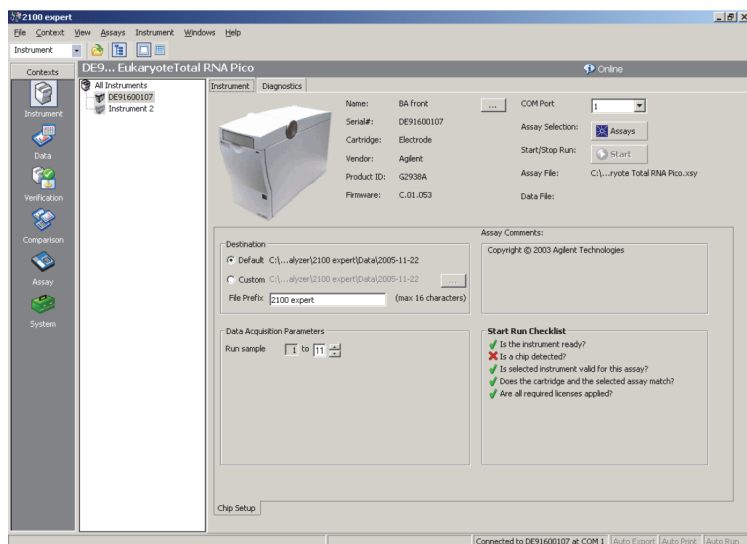
## Starting the 2100 Expert Software

To start the software:

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



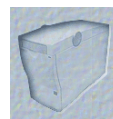
The screen of the software appears in the **Instrument** context. The icon in the upper part of the screen represents the current instrument/PC communication status:



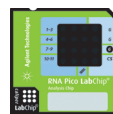
Lid closed, no chip or chip empty



Lid open



Dimmed icon: no communication



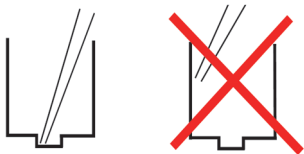
Lid closed, chip inserted, RNA or demo assay selected

- 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 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.
- 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. 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, microcentrifuge tubes and water.
- It is recommended to heat denature all RNA samples and RNA ladder before use for 2 minutes and 70 °C (once) and keep them on ice ([“Preparing the RNA Ladder after Arrival”](#) on page 13).
- 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 and spin down afterwards.
- Use a new syringe and electrode cleaners with each new kit.



## 4 Essential Measurement Practices

### Starting the 2100 Expert Software

- 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.



## Preparing the RNA Ladder after Arrival

For proper handling of the ladder, following steps are necessary:

### CAUTION

To avoid RNase contamination and repetitive freeze/thaw cycles, the RNA ladder must be aliquoted in RNase-free vials.

→ Tests have shown that some plastic vial types can bind RNA on their surface. This can have an effect on RNA ladder concentration which affects the ladder identification and the quantitation of the sample. Eppendorf Safe-Lock PCR clean and Eppendorf DNA LoBind 0.5ml Microcentrifuge tubes were successfully tested for RNA ladder aliquoting.

---

**1** After reagent kit arrival, spin ladder down. The ladder can be ordered separately (reorder number 5067-1535).

### NOTE

In case that the ladder vial type is not compatible with your heating block, transfer the entire volume (10  $\mu$ l) to a recommended RNase-free vial.

**2** Heat denature the ladder for 2 min at 70 °C.

**3** Immediately cool the vial on ice.

**4** Add 90  $\mu$ L of RNase-free water and mix thoroughly.

**5** Prepare aliquots in recommended 0.5 mL RNase-free vials with the required amount for typical daily use.

**6** Store aliquots at -70 °C.

**7** Before use, thaw ladder aliquots and keep them on ice (avoid extensive warming upon thawing process).





## Agilent RNA 6000 Pico Assay Protocol

### Agilent 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.

#### NOTE

If you use the RNA 6000 Pico kit for the first time, you must read these detailed instructions. If you have some experience, you might want to use the *Agilent RNA 6000 Pico Quick Start Guide*.

### Cleaning the Electrodes before Running Assays

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

#### NOTE

To prevent contamination problems, it is strongly recommended to use a dedicated electrode cartridge for RNA assays.

- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ l of fresh RNase-free water.
- 2 Open the lid and place the 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 to allow the water on the electrodes to evaporate before closing the lid.



## Preparing the Gel

- 1 Allow all reagents to equilibrate to room temperature for 30 minutes before use.
- 2 Place 550  $\mu$ l of RNA 6000 Pico gel matrix (red ●) into the top receptacle of a spin filter.

### NOTE

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

---

- 3 Place the spin filter in a microcentrifuge and spin for 10 minutes at 1500  $g \pm 20\%$  (for Eppendorf microcentrifuge, this corresponds to 4000 rpm).
- 4 Aliquot 65  $\mu$ l filtered gel into 0.5 ml RNase-free microcentrifuge 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

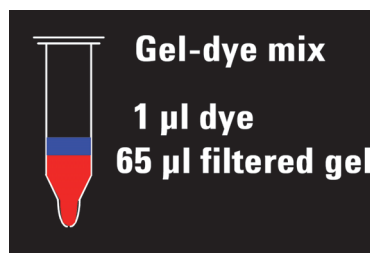
### WARNING

#### 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 solutions 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 15).
- 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

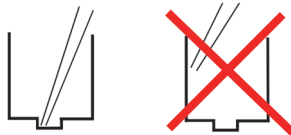
Before loading the gel-dye mix, make sure that the base plate of the chip priming station is in position (C) and the adjustable clip is set to the highest position. Refer to “[Setting up the Chip Priming Station](#)” on page 8 for details.

- 1 Allow the gel-dye mix to equilibrate to room temperature for 30 minutes before use. Protect the gel-dye mix from light during this time.
- 2 Take a new RNA chip out of its sealed bag.
- 3 Place the chip on the chip priming station.
- 4 Pipette 9.0  $\mu$ l of the gel-dye mix at the bottom of the well marked  and dispense the gel-dye mix.

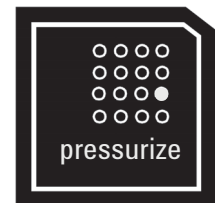


### NOTE

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.



- 5 Set the timer to 30 seconds, make sure that the plunger is positioned at 1 ml and then close the chip priming station. The lock of the latch will click when the chip priming station is closed correctly.
- 6 Press the plunger of the syringe down until it is held by the clip.
- 7 Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.
- 8 Visually inspect that the plunger moves back at least to the 0.3 ml mark.
- 9 Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.



## 6 Agilent RNA 6000 Pico Assay Protocol

### Loading the RNA 6000 Pico Conditioning Solution and Marker

- 10 Open the chip priming station.
- 11 Pipette 9.0  $\mu\text{l}$  of the gel-dye mix in each of the wells marked **G**

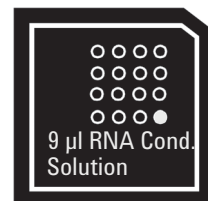


#### NOTE

Please discard the remaining vial with gel-dye mix.

## Loading the RNA 6000 Pico Conditioning Solution and Marker

- 1 Pipette 9  $\mu\text{l}$  of the RNA 6000 Pico conditioning solution (white  $\circ$ ) into the well marked CS.
- 2 Pipette 5  $\mu\text{L}$  of the RNA 6000 Pico marker (green  $\bullet$ ) into the well marked with a ladder (symbol  $\text{L}$ ) and each of the 11 sample wells.



#### NOTE

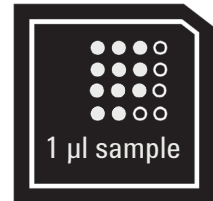
Do not leave any wells empty or the chip will not run properly. Add 5  $\mu\text{L}$  of the RNA marker (green  $\bullet$ ) plus 1  $\mu\text{L}$  of deionized water to each unused sample well.

## Loading the Diluted Ladder and Samples

### NOTE

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

- 1 To minimize secondary structure, you may heat denature (70 °C, 2 minutes) the samples before loading on the chip.
- 2 Before use, thaw ladder aliquots and keep them on ice (avoid extensive warming upon thawing process).



### NOTE

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

- 3 Pipette 1 µl of the diluted RNA 6000 Pico ladder (prepared as described in “[Preparing the RNA Ladder after Arrival](#)” on page 13) into the well marked with the ladder symbol
- 4 Pipette 1 µl of each sample into each of the 11 sample wells.
- 5 Place the chip horizontally in the adapter of the IKA vortex mixer and make sure not to damage the buldge that fixes the chip during vortexing.



If there is liquid spill at the top of the chip, carefully remove it with a tissue.

### CAUTION

Wrong vortexing speed

If the vortexing speed is too high, liquid spill that disturbs the analysis may occur for samples generated with detergent containing buffers.

→ Reduce vortexing speed to 2000 rpm!

- 6 Vortex for 60 seconds at 2400 rpm.
- 7 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. Best results are achieved for RNA samples which are dissolved in deionized and RNase-free water. Avoid genomic DNA contamination by including DNase treatment in the preparation protocol.

## Inserting a Chip in the Agilent 2100 Bioanalyzer

- 1 Open the lid of the Agilent 2100 bioanalyzer.
- 2 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.
- 3 Place the chip carefully into the receptacle. The chip fits only one way.

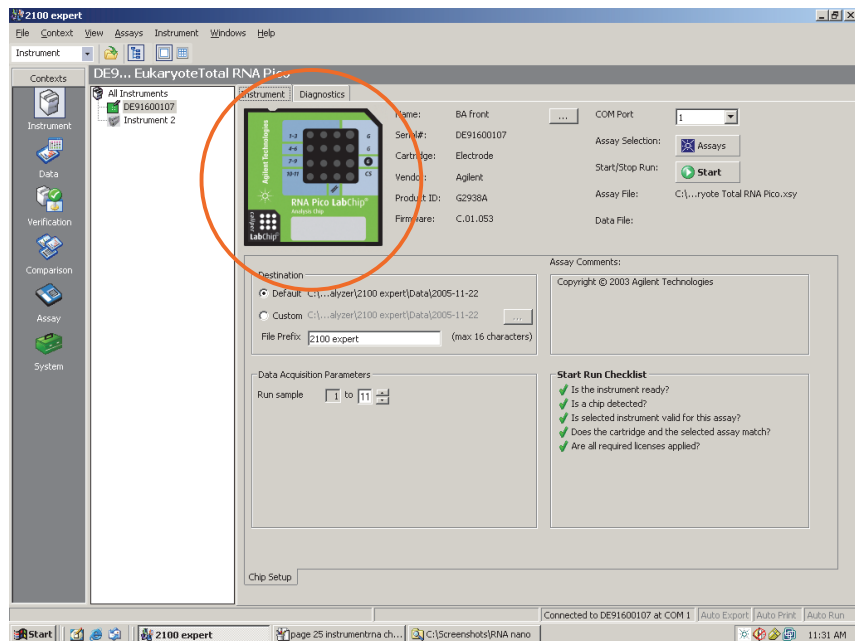
### CAUTION

Sensitive electrodes and liquid spills

Forced closing of the lid may damage the electrodes and dropping the lid may cause liquid spills resulting in bad results.

→ Do not use force to close the lid and do not drop the lid onto the inserted chip.

- 4 Carefully close the lid. The electrodes in the cartridge fit into the wells of the chip.
- 5 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.

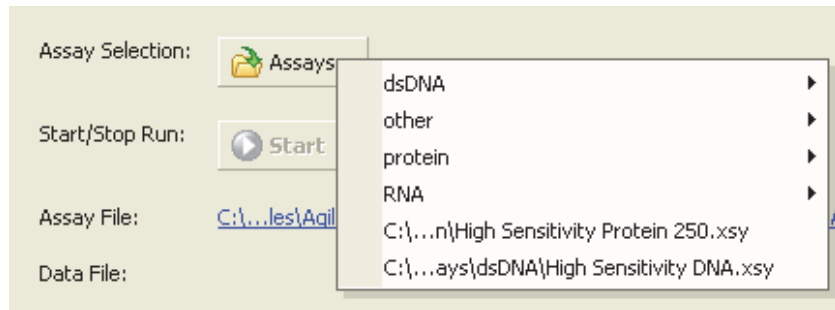


## Starting the Chip Run

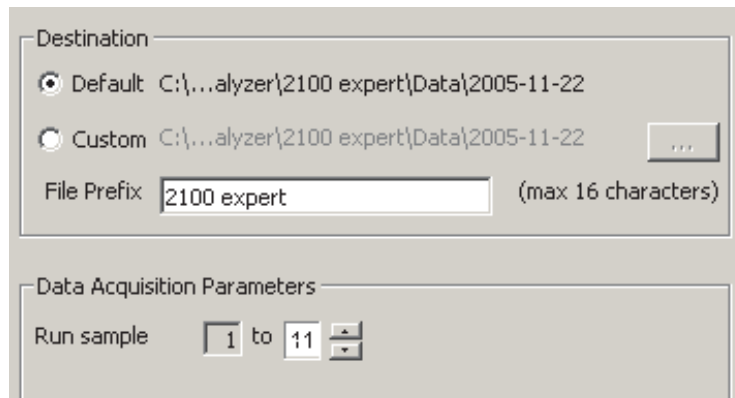
**NOTE**

Please note that the order of executing the chip run may change if the Agilent Security Pack software (only applicable for Agilent 2100 expert software Revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 expert software.

- 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.

## 6 Agilent RNA 6000 Pico Assay Protocol

### Starting the Chip Run

- 3 To enter sample information like sample names and comments, complete the sample name table.

	Sample Name	Sample Comment	Status	Observation	Result Label	Result Color
▶	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					
12	Sample 12					

Chip Lot #      Reagent Kit Lot #

Chip Comments :

Sample Information    Study Information

Import...      Export...

- 4 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.



### CAUTION

#### Contamination of electrodes

Leaving the chip for a period longer than 1 hour (e.g. over night) in the Bioanalyzer may cause contamination of the electrodes.

→ Immediately remove the chip after a run.

- 5 After the chip run is finished, remove the chip from the receptacle of the bioanalyzer and dispose it according to good laboratory practices.

## Cleaning Electrodes after a RNA 6000 Pico Chip Run

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

**NOTE**

Use a new electrode cleaner with each new kit.

**CAUTION**

Leak currents between electrodes

Liquid spill may cause leak currents between the electrodes.

→ Never fill too much water in the electrode cleaner.

- 1 Slowly fill one of the wells of the electrode cleaner with 350  $\mu$ l of fresh RNase-free water.
- 2 Open the lid and place the electrode cleaner in the Agilent 2100 bioanalyzer.
- 3 Close the lid and leave it closed for 30 s.
- 4 Open the lid and remove the electrode cleaner.
- 5 Wait another 30 seconds to allow 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 RNaseZap® onto electrodes and soak for 1 minute.
- 3** Gently brush the electrodes.
- 4** Rinse electrodes thoroughly with RNase-free water.

---

**NOTE**

The rinsing step is very important and needs special attention as residual RNaseZap may alter the results creating ghost peaks.

---

- 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, the cartridge may still be wet. Repeat the drying procedure.

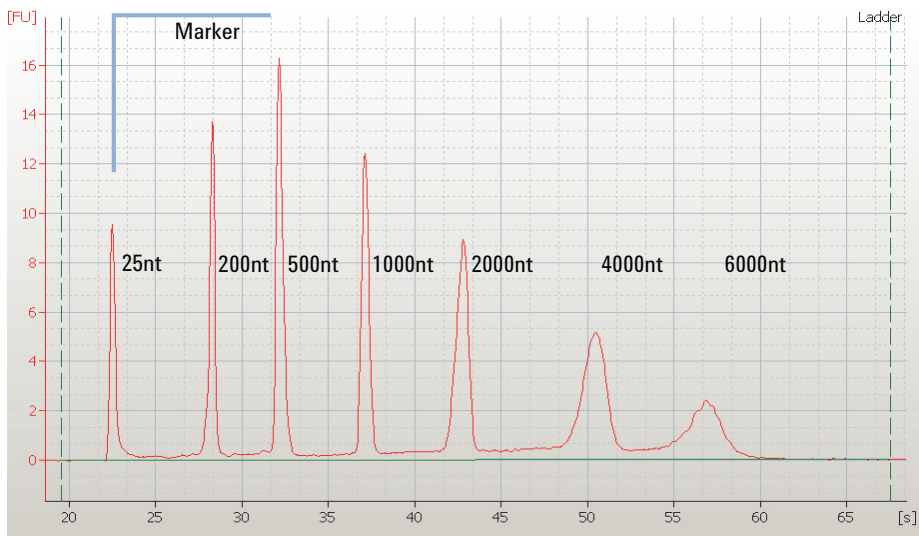
---



## Checking Your Agilent RNA 6000 Pico Assay Results

### RNA 6000 Pico Ladder Well Results

To check the results of your run, select the Gel or Electropherogram tab in the *Data* context. The electropherogram of the ladder well window should resemble those as shown below.



**Figure 1** RNA 6000 Pico ladder

Major features of a successful ladder run are:

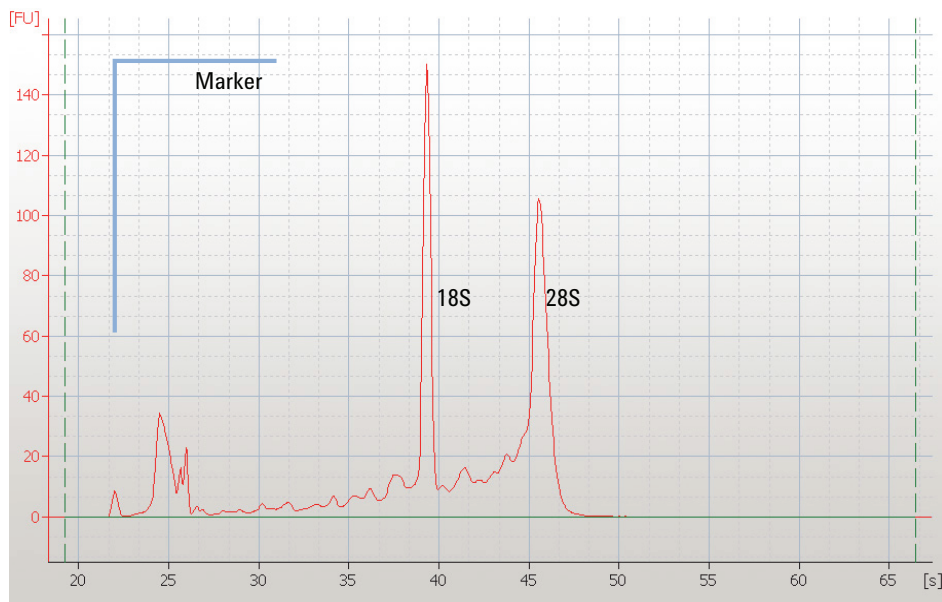
- 1 marker peak
- 6 RNA peaks (2100 expert software calls for 5 first ladder peaks only)
- All 7 peaks are well resolved
- Correct peak size assignment in electropherogram

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.



## RNA 6000 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.



**Figure 2** RNA peaks of a successful sample run

Major features for a successful total RNA run are:

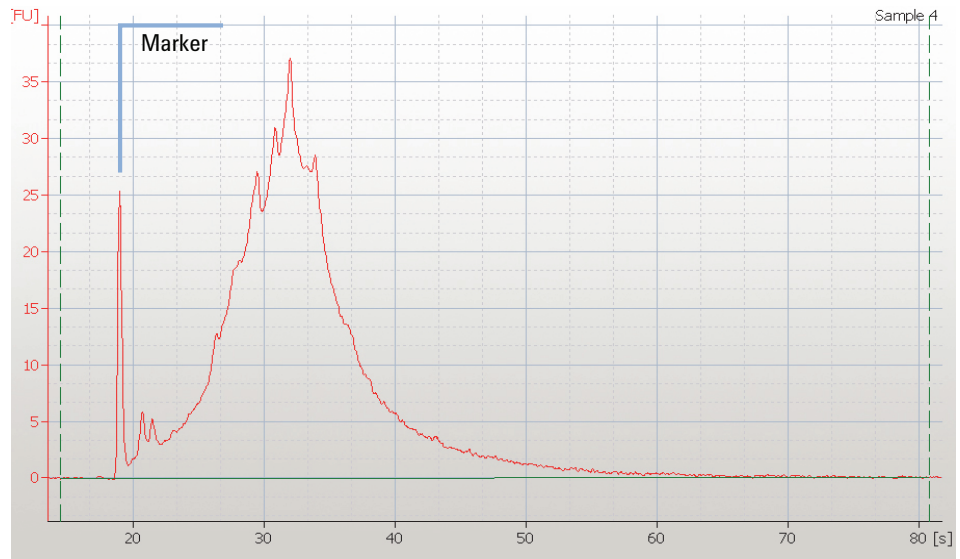
- 1 marker peak
- 2 ribosomal peaks (with successful sample preparation)

By selecting the *Results* sub-tab, values for the calculated RNA concentration, the ribosomal ratio and the RNA Integrity Number (RIN), implemented with 2100 expert software version B.02.02 or higher, 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.



**Figure 3** mRNA peaks of a successful sample run

Major features for a successful mRNA run are:

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

## In this book

You find the procedures to analyze RNA samples with the Agilent RNA 6000 Pico reagent kit and the Agilent 2100 Bioanalyzer instrument.

- Agilent RNA 6000 Pico Kit
- Required Equipment for RNA 6000 Pico Assay
- Setting up the Assay Equipment and Bioanalyzer
- Essential Measurement Practices
- Preparing the RNA Ladder after Arrival
- Agilent RNA 6000 Pico Assay Protocol
- Checking Your Agilent RNA 6000 Pico Assay Results

© Agilent Technologies 2001, 2003-2008, 2013

Printed in Germany  
11/2013



G2938-90046  
Rev. B



**Agilent Technologies**