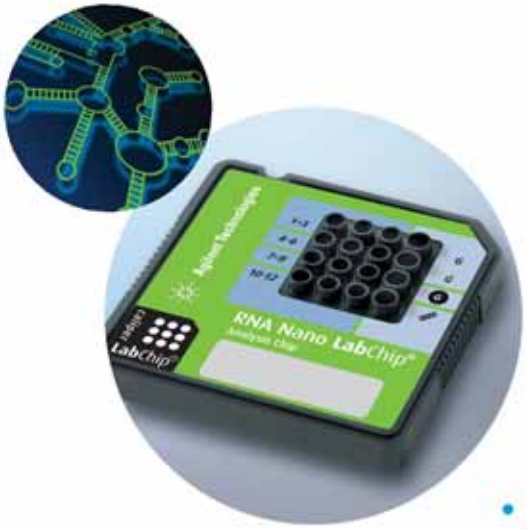
PROTOCOL CHANGE

Vortexer speed now 2400 rpm



Agilent RNA 6000 Nano Kit Guide

Agilent Technologies

Notices

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WARNING

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Contents

Agilent RNA 6000 Nano Kit

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

Agilent RNA 6000 Nano Chips	**Agilent RNA 6000 Nano Reagents (reorder number 5067-1512) & Supplies**
25 RNA Nano Chips	(yellow) Agilent RNA 6000 Ladder (reorder number 5067-1529)
2 Electrode Cleaners	(blue) RNA Nano Dye Concentrate*
Tubes for Gel-Dye Mix	(green) Agilent RNA 6000 Nano Marker (2 vials)
30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free)	(red) Agilent RNA 6000 Nano Gel Matrix (2 vials)
Syringe Kit	4 Spin Filters
1 Syringe	
Tubes for Gel-Dye Mix	
30 Safe-Lock Eppendorf Tubes PCR clean (DNase/RNase free) for gel-dye mix	

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Physical Specifications

Type	**Specification**
Analysis run time	30 minutes
Number of samples	12 samples/chip
Sample volume	1 µl
Kit stability	4 months (Storage temperature see individual box!)

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
Quantitation accuracy	20 % CV (for ladder as sample)	20 % CV (for ladder as sample)
Buffer compatibility*	100 mM Tris or 125 mM NaCl or 15 mM $MgCl_2$	100 mM Tris or 125 mM NaCl or 15 mM $MgCl_2$
Reproducibility of quantitation	10 % CV	10 % CV

Required Equipment for RNA 6000 Nano Assay

Equipment Supplied with the Agilent 2100 Bioanalyzer

- Chip priming station (reorder number 5065-4401)
- IKA vortex mixer
- 16-pin bayonet electrode cartridge (reorder number 5065-4413)

Additional Material Required (Not Supplied)

- RNaseZAP® recommended for electrode decontamination (Ambion, Inc. cat. no. 9780)
- RNase-free water
- Pipettes (10 µl and 1000 µl) with compatible tips (RNase-free, no filter tips, no autoclaved tips)
- 1.5 ml microcentrifuge tubes (RNase-free)
- Microcentrifuge (≥ 1300 g)
- Heating block or water bath for ladder/sample denaturation

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

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 DNA 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 Nano 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:

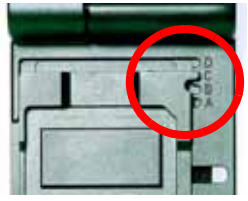
   a Unscrew the old syringe from the lid of the chip priming station.

   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 MS2-S8/MS2-S9

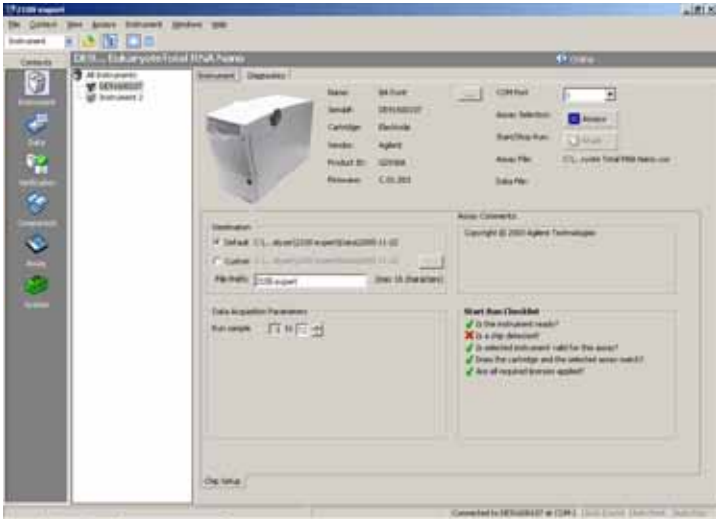
To set up the vortex mixer, adjust the speed knob to 2400 rpm.

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

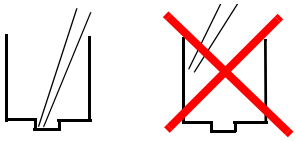
Dimmed icon: no communication

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 and samples to equilibrate to room temperature for 30 minutes before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. The dye decomposes when exposed to light and this reduces the signal intensity.
- Always insert the pipette tip to the bottom of the well when dispensing the liquid. Placing the pipette at the edge of the well may lead to poor results.



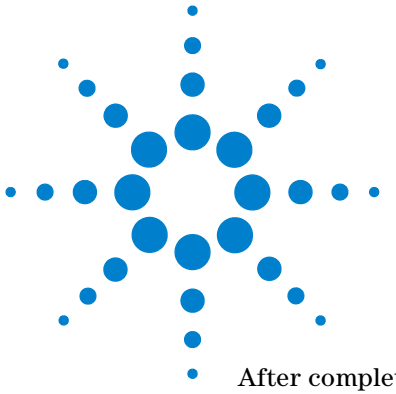
- Always wear gloves when handling RNA and use RNase-free tips, microfuge tubes and water.
- It is recommended to heat denature all RNA samples and RNA ladder before use ("Preparing the RNA Ladder after Arrival" on page 12).
- Do not touch the Agilent 2100 bioanalyzer during analysis and never place it on a vibrating surface.
- Always vortex the dye concentrate for 10 seconds before preparing the gel-dye mix.
- Use a new syringe and electrode cleaners with each new kit.

- Use loaded chips within 5 minutes. Reagents might evaporate, leading to poor results.
- To prevent contamination (e.g. RNase) problems, it is strongly recommended to use a dedicated electrode cartridge for RNA assays.
- Perform the following RNase decontamination procedure for the electrodes on a daily basis before running any assays.

Preparing the RNA Ladder after Arrival

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

1. After reagent kit arrival, pipette the ladder in RNase-free vial.
   The ladder can be ordered separately (reorder number 5067-1529).
2. Heat denature it for 2 min at 70 °C.
3. Immediately cool down the vial on ice.
4. Prepare aliquots in RNase-free vials with the required amount for a typical daily use.
5. Store aliquots at -70°C.
6. Before use, thaw ladder aliquots and keep them on ice (avoid extensive warming upon thawing process).



Agilent RNA 6000 Nano Assay Protocol

After completing the initial steps in "Setting up the Assay Equipment and Bioanalyzer" on page 6, 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 kit for the first time, you must read these detailed instructions. If you have some experience, you might want to use the *Agilent RNA 600 Nano Quick Start Guide*.

Decontaminating the Electrodes

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

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 25 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.

Preparing the Gel

1 Allow all reagents to equilibrate to room temperature for 30 minutes before use.

2 Place 550 µl of Agilent RNA 6000 Nano gel matrix (red ●) into the top receptacle 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 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

Handling DMSO

Kit components contain DMSO. Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.

⇒Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.

⇒Handle the DMSO stock solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

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 RNA 6000 Nano dye concentrate (blue ●) to a 65 µl aliquot of filtered gel (prepared as described in "Preparing the Gel" on page 14).
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.

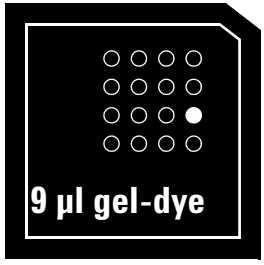
NOTE

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.

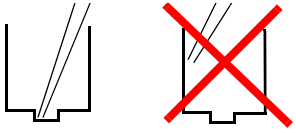
Loading the Gel-Dye Mix

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 top position. Refer to "Setting up the Chip Priming Station" on page 7 for details.

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



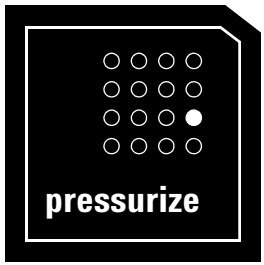
5. Set the timer to 30 seconds, make sure that the plunger is positioned at 1 ml and then close the chip priming station. The lock of the latch will click when the Priming Station is closed correctly.

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.

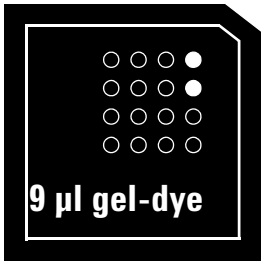
8 Visually inspect that the plunger moves back at least to the 0.3 ml mark.

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



10 Open the chip priming station.

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



NOTE Please discard the remaining vial with 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. Unused wells must be filled with 5 µl of the RNA 6000 Nano marker (green ●) plus 1 µl of the buffer in which the samples are diluted.

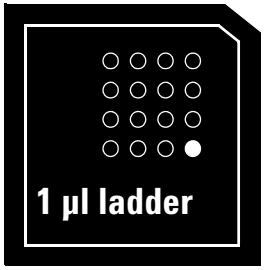
Loading the Ladder and Samples

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

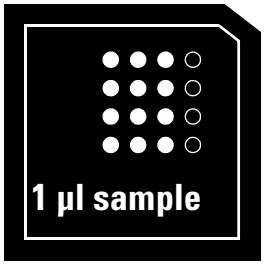
1 Before use, thaw ladder aliquots and keep them on ice (avoid extensive warming upon thawing process)

2 To minimize secondary structure, heat denature (70 °C, 2 minutes) the samples before loading on the chip.

3 Pipette 1 µl of the RNA ladder into the well marked with the ladder symbol .



4 Pipette 1 µl of each sample into each of the 12 sample wells.



CAUTION *Wrong vortexing speed*

If the vortexing speed is too high, liquid spill that disturbs the analysis may occur .

⇒Reduce vortexing speed to 2000 rpm!

5 Set the timer to 60 seconds.

6 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.

7 Vortex for 60 seconds at 2400 rpm.

8 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 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 8 for details.

3 Place the chip carefully into the receptacle. The chip fits only one way.

CAUTION

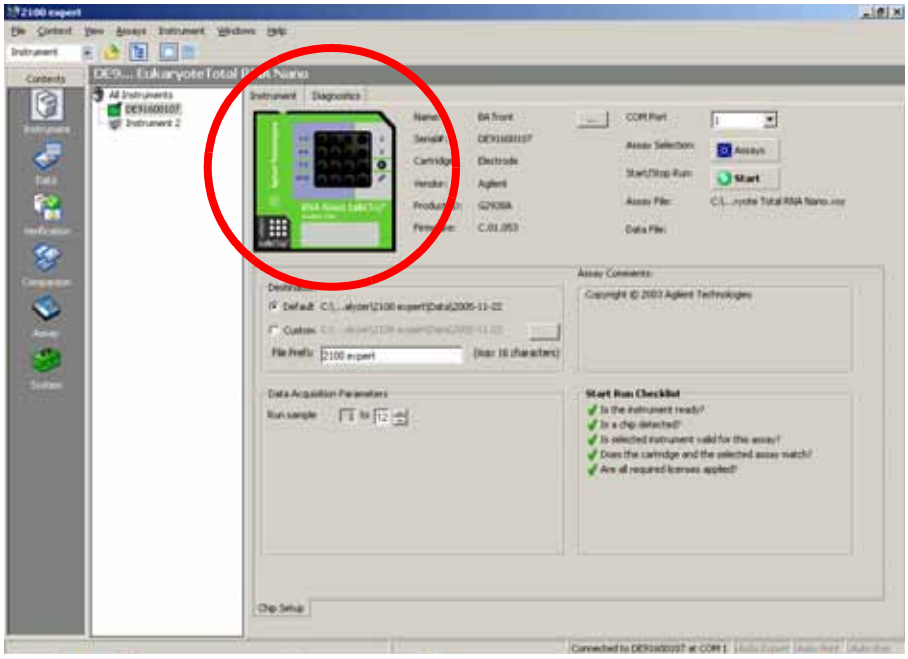
Sensitive electrodes and liquid spills

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.

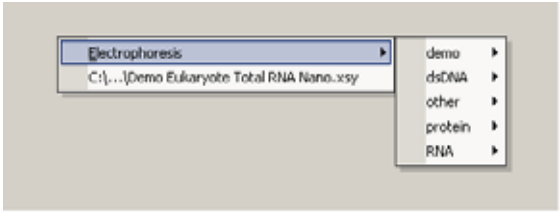
5 The 2100 expert software screen shows that you have inserted a chip and closed the lid by displaying the chip icon at the top left of the *Instrument* context.



Starting the Chip Run

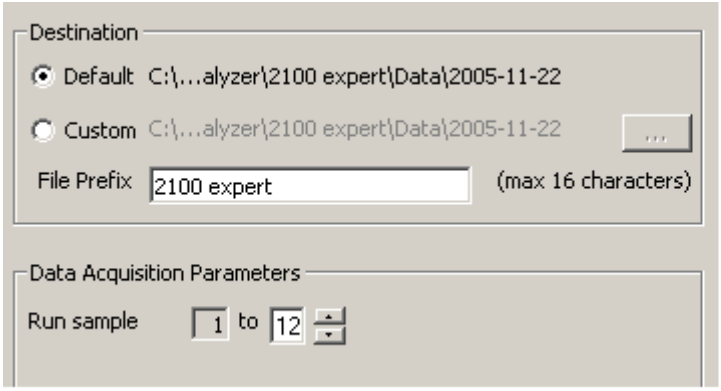
NOTE Please note that the order of executing the chip run may change if the Agilent Security Pack software (only applicable for Agilent 2100 expert software Revision B.02.02 and higher) is installed. For more details please read the 'User's Guide' which is part of the Online Help of your 2100 expert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

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



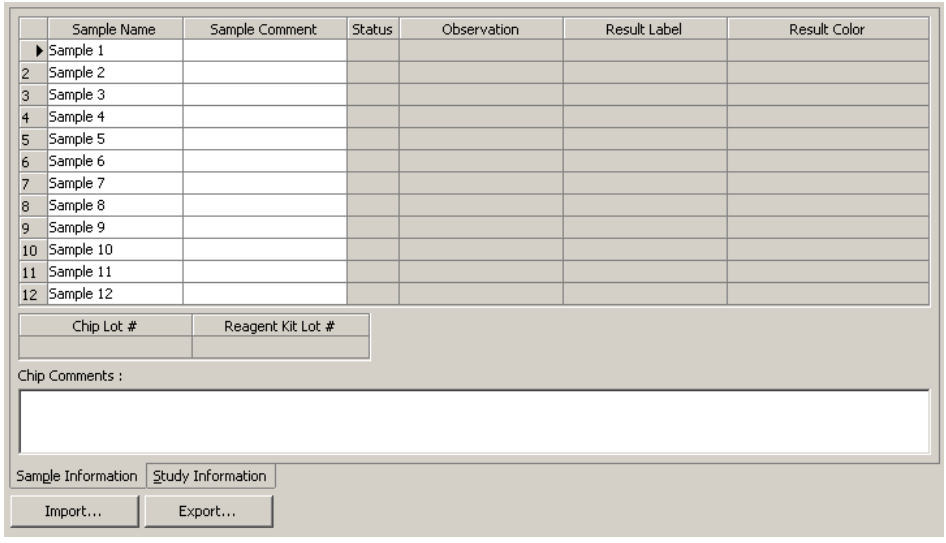
2 Accept the current *File Prefix* or modify it.

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.



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 *Assay* context and select the *Chip Summary* tab. Complete the sample name table.



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

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

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.

Cleaning up after a RNA 6000 Nano 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 (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 might 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 µl 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 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 before closing the lid.

NOTE After 5 chip runs, empty and refill the electrode cleaner.

After 25 chip runs, replace the used electrode cleaner by a new one.

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 *2100 Expert Maintenance and Troubleshooting Guide* which is part of the Online Help of the 2100 bioanalyzer software.

Checking Your Agilent RNA 6000 Nano Assay Results

RNA 6000 Nano 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 shown below.

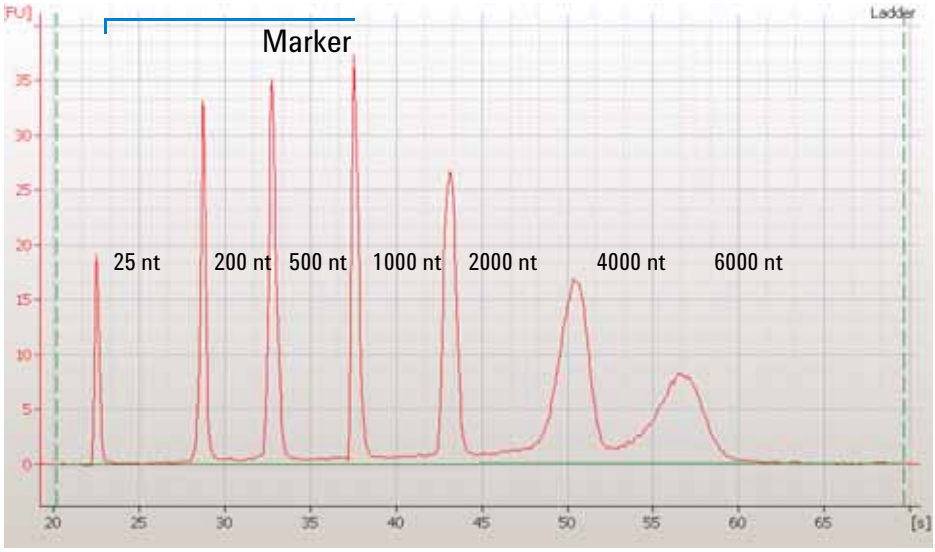


Figure 1 RNA 6000 Nano ladder

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 the 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 Nano 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.

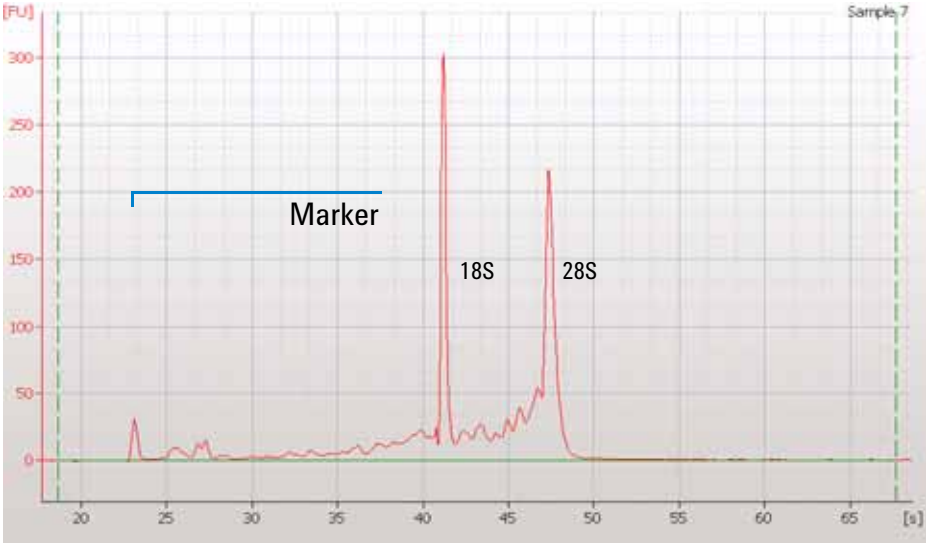


Figure 2 RNA peaks of a successful sample run

Major features of 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.

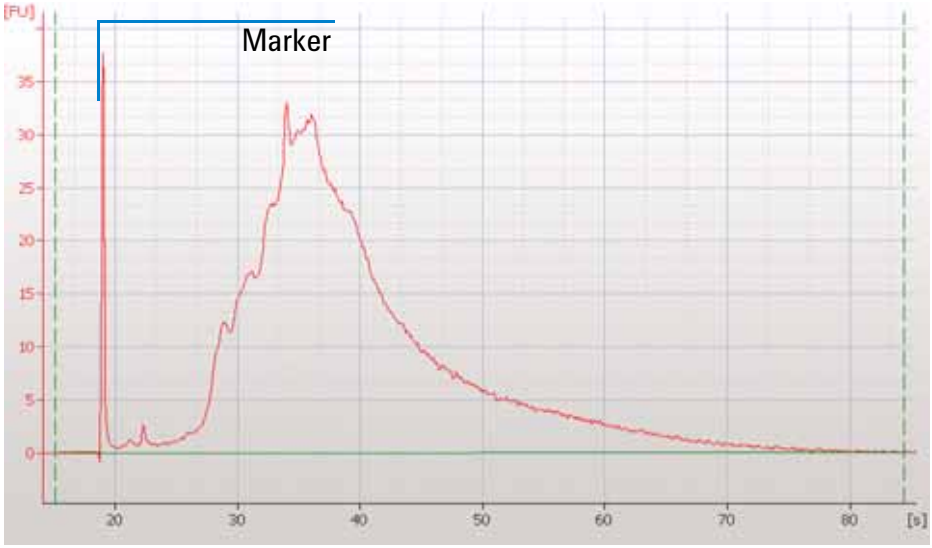


Figure 3 mRNA peaks of a successful sample run

Major features of 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)

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In This Book

you find the procedures to analyze RNA samples with the Agilent RNA 6000 Nano reagent kit and the Agilent 2100 expert bioanalyzer.

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