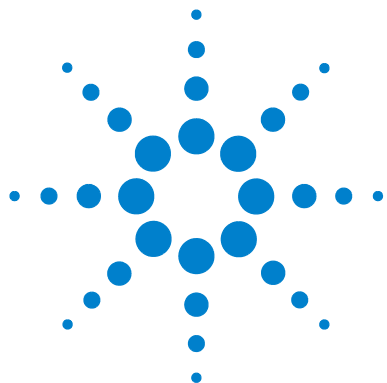




Agilent Small RNA Kit Quick Start Guide

Agilent Small RNA Kit (reorder number 5067-1548)

RNA Chips

25 Small RNA Chips
3 Electrode Cleaners

Syringe Kit

1 Syringe

Reagents (reorder number 5067-1549) & Supplies

● (blue) Small RNA Dye Concentrate*
● (green) Small RNA Marker (4 vials)
○ (white) Small RNA Conditioning Solution
● (red) Small RNA Gel Matrix (2 vials)
● (yellow) Small RNA Ladder (reorder number 5067-1550)
2 Spin Filters + 30 tubes for gel-dye mix

*) "This product is provided under an agreement between Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation) and Agilent Technologies. The manufacture, use, sale or import of this product may be subject to one or more of U.S. patents, pending applications, and corresponding international equivalents, owned by Molecular Probes, Inc. The purchaser has the non-transferable right to use the product to detect protein and/or nucleic acids in microfluidics analysis systems for one or more of the subfields of research, development, quality control, forensics, environmental analysis, biodefense, food safety testing, veterinary diagnostics, or human diagnostics, according to use indicated on the product label or accompanying product literature. For information on obtaining a license, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402-9132. Tel: (541) 465-8300. Fax: (541) 335-0354."

Assay Principles

Agilent RNA kits contain chips and reagents designed for analysis of small RNA fragments. Each small RNA 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. Agilent RNA kits are designed for use with the Agilent 2100 bioanalyzer only.

Assay Kits

Agilent Small RNA Kits are designed for the analysis of total RNA (eukaryotic and prokaryotic) and samples enriched for small nucleic acids. The complete Small RNA Assay kit guide can be found in the Online Help of the 2100 expert software.

Storage Conditions

- Freeze the unused Small RNA Ladder at -20 °C and keep all reagents and reagent mixes refrigerated at 4°C when not in use to avoid poor results caused by reagent decomposition.
- Protect Marker solution (contains a fluorescent dye), dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

Equipment Supplied with the Agilent 2100 Bioanalyzer

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



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Piglet Small RNA Kit Quick Start Guide

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)
- 0.5 ml and 1.5 ml microcentrifuge tubes (RNase-free, Eppendorf Safe-Lock PCR clean or Eppendorf DNA LoBind microcentrifuge tubes are highly recommended.)
- Microcentrifuge (≥ 1300 g)
- Heating block or water bath for ladder/sample preparation
- Mandatory: bayonet electrode cartridge (reorder number 5065-4413)
- 2100 Expert SW rev B.02.05 or higher.

Sample Preparation

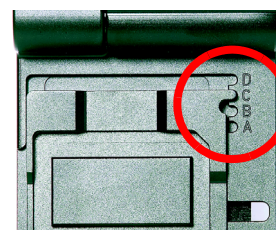
- Prepare RNA samples in deionized water. To analyze RNA, total RNA in sample must be between 1-100 ng/µl. Recommended concentration of purified small RNA samples (≤ 150 nt) is 1-20 ng/µl. If concentration of your particular sample is above this range, dilute with RNase-free water.

Physical Specifications		Analytical Specifications	
Type	Specification	Specification	Small RNA Assay
Analysis run time	30 minutes	Analysis range	6-150 nt
Number of samples	11 samples/chip	Quantitation reproducibility	25 % CV (for ladder as sample)
Sample volume	1 µl	Quantitative range	50-2000 pg/µl for purified miRNA (in water)
Kit stability	4 months (Storage temperature see individual box!)	Max. Buffer concentration*	10 mM Tris and 0.1 mM EDTA
		Sensitivity (Signal/Noise > 3)	50 pg/µl (for 40 nt fragment of diluted ladder)

*)Due to the high sensitivity of the assay, different ions and higher salt concentrations might influence the performance of the assay.

Setting up the Chip Priming Station

- 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 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.



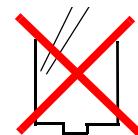
3 Adjust the syringe clip:

- a Release the lever of the clip and slide it up to the lowest position.



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.
- Allow all reagents to equilibrate to room temperature for 30 minutes before use.
- Keep all reagents and reagent mixes refrigerated at 4°C when not in use.
- Protect marker solution, 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.
- The gel matrix is very viscous! Proper pipetting and mixing requires special attention.
- 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.
- 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. Spin down afterwards.
- Use a new syringe and electrode cleaners with each new kit.
- Use loaded chips within 5 minutes after preparation. 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.



Agilent Small RNA Assay Protocol - Edition April 2007

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.

Preparing the Small RNA Ladder after Arrival

- 1 After reagent kit arrival spin the ladder down.
- 2 Heat denature it for 2 min at 70 °C.
- 3 Immediately cool down the vial on ice.
- 4 Prepare aliquots in 0,5 ml RNase-free vials (Eppendorf Safe-lock PCR clean or Eppendorf DNA LoBind microcentrifuge tubes) with the required amount for a typical daily use.
- 5 Store aliquots at -70 °C.
- 6 Before use, thaw ladder aliquots on ice.

Agilent Small RNA Assay Protocol - Edition March 2008

Preparing the Gel

- 1 Pipette the complete volume (approx. 650 µl) of the Small RNA gel matrix (red ●) into a spin filter.
- 2 Centrifuge at 10000 g ± 20 % for 15 minutes at room temperature. Use filtered gel within 4 weeks.

Preparing the Gel-Dye Mix

- 1 Allow all reagents to equilibrate to room temperature for 30 min.
- 2 Vortex Small RNA dye concentrate (blue ●) for 10 seconds, spin down.
- 3 Pipette 2 µl of dye into 0.5 ml RNase free microtubes (provided with the kit).
- 4 Add 40 µl of filtered gel (gel is very viscous, careful pipetting is highly recommended).
- 5 Mix using pipetting, or by flipping over and flicking the vial several times until the dye is distributed equally. Visually inspect that the mix is homogeneous.
- 6 Spin tube at 13000 g for 10 minutes at room temperature.



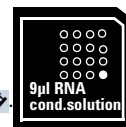
Loading the Gel-Dye Mix

- 1 Put a new Small RNA chip on the chip priming station.
- 2 Pipette 9.0 µl of gel-dye mix in the well marked G.
- 3 Close the chip priming station.
- 4 Press plunger until it is held by the clip.
- 5 Wait for exactly 60 seconds then release clip.
- 6 Visually inspect that the plunger moves back at least to the 0.3 ml mark.
- 7 Wait for 5 seconds, then slowly pull back the plunger to the 1ml position.
- 8 Pipette 9.0 µl of gel-dye mix in the wells marked G.
- 9 Discard the remaining gel-dye mix.



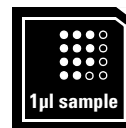
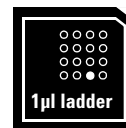
Loading the Small RNA Conditioning Solution and Marker

- 1 Slowly pipette 9.0 µl of the Small RNA Conditioning Solution (white ○) in the well marked CS.
- 2 Pipette 5 µl of Small RNA marker (green ●) in all 11 sample wells and in the well marked M.



Loading the Ladder and Samples

- 1 Pipette 1 µl of the heat denatured and aliquoted ladder in the well marked L.
- 2 Pipette 1 µl of sample in each of the 11 sample wells. Pipette 1 µl of Small RNA Marker (green ●) in each unused sample well.
- 3 Put the chip in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
- 4 Run the chip in the Agilent 2100 bioanalyzer within 5 min.



Technical Support Please visit our support web page <http://www.agilent.com/chem/contactus> to find contact information of your local Contact Center. In Japan: email_japan@agilent.com.

Further Information Visit Agilent Technologies' unique Lab-on-a-Chip web site. It is offering useful information, support and current developments about the products and the technology: <http://www.agilent.com/chem/labonachip>.



Part Number: G2938-90092
Edition 03/2008
Printed in Germany

© Agilent Technologies, Inc. 2007, 2008
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76337 Waldbronn, Germany