RNA 004 v.003

Assessing the quality of RNA using the Agilent 2100 Bioanalyzer

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1.0 Title

Assessing the Quality of RNA Using the Agilent 2100 Bioanalyzer

2.0 Purpose

To prepare and run Agilent Bioanalyzer 2100 in order to assess the quality of RNA before processing it for Gene Expression Profiling

3.0 Definitions and Abbreviations

RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
PBS	Phosphate Buffered saline

4.0 Equipment / Reagents

Equipment

	Name	Vendor	Catalog No.
4.1	Agilent 2100 Bioanalyzer	Agilent Technologies Inc.	
4.2	Chip priming station	Agilent Technologies Inc.	5065-4401
4.3	IKA Vortex	IKA	Model MS2-S8 / MS2-S9
4.4	Pipettes	Can be chosen by the Lab	
4.5	0.5 ml and 1.5 ml Tubes	Can be chosen by the Lab	
4.6	Micro-centrifuge		
4.7	Heating Block		
4.8	16-pin Electrode Cartridge	Agilent Technologies, Inc.	5065-4413

Reagents

4.9	RNA 6000 Nano LabChip Kit	Agilent Technologies, Inc.	5065-4476

4.10	RNA 6000 Nano reagents and Supplies	Agilent Technologies, Inc.	5065-4475
4.11	RNA 6000 ladder	Ambion, Inc.	7152
4.12	RNaseZAP®	Ambion, Inc.	9780
4.13	RNase-free water		

5.0 Procedures

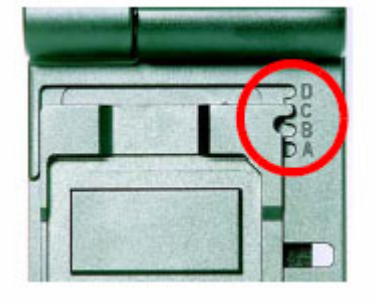
Note: This protocol should serve as a reference ONLY. For more details please refer to the Reagent Kit Guide "RNA 6000 Nano Assay" (Agilent Technologies Inc., part No. G2941-90126)

5.1 Setting up all necessary equipment

5.1.1 Setting up the chip primining station

5.1.1.1 **Replace the Syringe with each new Reagent Kit!**

5.1.1.2 Before use, make sure that the base-plate is adjusted properly.



5.1.1.3 Before use, make sure that the Syringe Clip is properly adjusted.

5.1.2 Setting up Bioanalyzer

5.1.2.1 Open the lid of the instrument and make sure that the electrode cartridge is in place.

5.1.2.2 Adjust the chip selector position to "1".

5.1.3 Setting up the Software

5.1.3.1 Double-click on the icon that opens the software for the Agilent 2100 Bioanalyzer.

5.2 Preparation of the Agilent 2100 Bioanalyzer

Note: the following decontamination procedure should be performed daily before running any assays.

5.2.1 Slowly fill a well of an electrode cleaning cartridge with 350 µl of RNA-Zap or RNAse Away.

5.2.2 Place the electrode cleaner into the Agilent Bioanalyzer 2100, close the lid and leave the cartridge in the machine for 1 minute.

5.2.3 After 1 minute, remove the electrode cleaner from the Bioanalyzer and store it for future use. This cartridge can be re-used for all the chips from the same kit.

5.2.4 Slowly fill a well of an electrode cleaning cartridge with 350 µl with Nuclease-free water.

5.2.5 Place electrode cleaner into the Bioanalyzer and leave it there for 10 seconds. After 10 seconds, remove the chip.

5.2.6 Keep the lid of the Bioanalyzer open for another 10 seconds and let it stay close for some time. This will allow the water to evaporate from the capillaries.

5.2.7 Between Bioanalyzer chips clean electrodes ONLY with the Nuclease free water cartridge. This will prevent any RNAse build up.

At the end of the Bioanalyzer session repeat steps 5.2.1 to 5.2.6.

5.3 Preparation of RNA 6000 Nano gel matrix

Note:

- **Keep all reagents and reagents mixes at 4˚ C when not in use**
- **Allow all reagents and samples to equilibrate to room temperature for 30 minutes before use**
- **Use loaded chips within 5 minutes**

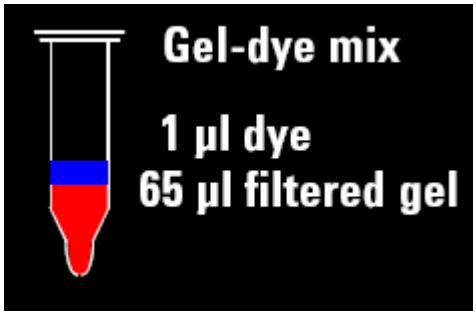
5.3.1 Place 550 µl of RNA 6000 Nano gel matrix on top of spin filter.

5.3.2 Spin at 1,500 g for 10 minutes.

5.3.3 Aliquot gel matrix into 65 µl samples.

5.3.4 Vortex RNA 6000 Nano Dye concentrate (blue cap).

5.3.5 Add 1 µl of Nano Dye concentrate to a 65 µl filtered gel matrix.



5.3.6 Cap the tube and vortex thoroughly.

5.3.7 Spin tube for 10 minutes at 13,000 g at RT. Use prepared mix within one day.

5.4 Sample preparation

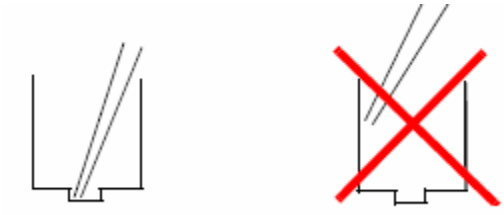
5.4.1 Denature samples at 70˚ C for 2 minutes. After 2 minutes spin shortly and then place on ice.

5.5 Preparing and running the Bioanalyzer chip

5.5.1 Place chip on the Chip Priming Station.

5.5.2 Load 9 ul of gel dye mix into the well marked and pressure it with syringe to spread into all of the capillaries and wait for **exactly** 30 seconds before releasing the clip. Add 9 µl of the gel to the other "Gel" wells (wells without a dot).

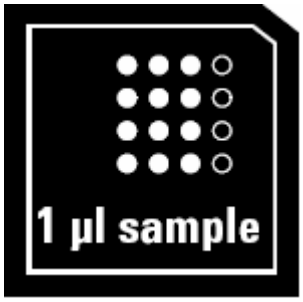
5.5.3 Please observe proper loading techniques.



5.5.4 Pipette 5.0 µl of the RNA 6000 Nano Marker (green top) into the well marked as "Ladder" and 5.0 µl into each of 12 sample wells. For each unused well add 6 µl of the Nano Marker (green top).

5.5.5 Load 1 µl of Ladder into "Ladder" well.

5.5.6 Load 1 µl of sample into each sample well.



5.5.7 Vortex the chip for 1 minute at 2400 rpm.

5.5.8 Load the chip into an Agilent Bioanalyzer 2100 within 5 minutes..

5.5.9 Run Agilent Bioanalyzer 2100.

6.0 Cleaning of the equipment

For cleaning instructions, please follow the steps listed in Section 5.2 (Preparation of the Agilent Bioanalyzer 2100).

6.1 Perform cleaning of the Agilent Bioanalyzer 2100 daily before and after any runs.

6.2 For equipment that is used on daily basis, please perform a more thorough cleaning.

6.2.1 Once a month use the toothbrush to clean the capillary's area inside of the machine.

6.2.2 Please refer to the 2100 Maintenance and Troubleshooting Guide provided with Agilent Bioanalyzer 2100 for further instructions on cleaning procedures.

REVISION HISTORY			
Rev	**Section**	**Type**	**Initials/Dates**
001	All	Scheduled SOP review and update.	SK / 09.09.05
002	SOP number	Changed SOP number from RNA_003 to RNA_04	SK / 09.09.05