

# BioMarkers: Agilent RNA 6k Nano Assay Protocol

Agilent RNA 6000 Nano Assay Protocol (Taken from Agilent booklet and amended with information from Agilent Engineers)

*Note: RNA 6000 Nano Assay Reagents are stored at 4C with the exception of the chips, which are stored at room temperature. The dye is light sensitive; thus the dye concentrate tube and gel-dye mix is wrapped in foil. After results are obtained, enter data in RNA Yields file under Bioanalyzer.*

## Preparing Reagents:

1. Allow reagents to come to room temperature over 30-60 minutes.
2. After reagents come to RT, vortex and centrifuge briefly to collect droplets.
3. If reusing gel-dye mix, vortex thoroughly and centrifuge at max speed for 15 minutes to get rid of microbubbles.
4. Thaw a ladder aliquot for each chip; also thaw samples if appropriate. Each chip can hold 12 samples for analysis. (Aliquots are stored in the Agilent Prep Box in –80°C).

## Preparing Gel Matrix:

1. Place 400ul of RNA Gel Matrix (red top tube) into the top receptacle of a spin filter provided with kit.
2. Place the spin filter in a microcentrifuge and spin at 3800 rpm for 10 minutes.
3. This Gel Matrix can be used up to one month when stored at 4C.

## Preparing Gel-Dye Mix:

1. Place 130ul of your **filtered RNA gel matrix** into an RNase free 1.5ml microcentrifuge tube and add 2ul of RNA dye concentrate.
2. Vortex thoroughly and spin at maximum speed in microcentrifuge for 15 minutes to get rid of microbubbles.
3. This solution must be protected from light: wrap in foil.
4. This solution can be used for one week when stored at 4C. When re-using, vortex thoroughly and centrifuge at max speed for 15 minutes to get rid of microbubbles.

## Ladder: (RNA 6000 ladder, Ambion Inc., Cat#7152)

1. To collect consistent data, the ladder is mixed with RNA 6000 NanoMarker in mass quantity and aliquoted. The ladder and aliquots are stored in the Agilent Prep box in the -80°C.
  - a. Denature the ladder at 70°C for 2 minutes and then immediately put it on ice.
  - b. Combine the ladder and NanoMarker in an RNase free centrifuge tube. Add 1ul of RNA ladder for every 5ul of NanoMarker. (Or 20 and 100 ul).
  - c. Vortex briefly and then centrifuge to collect droplets.
  - d. Aliquot 6.5ul into 0.5 microfuge tubes for storage at –80C. Stored in Agilent Prep Box.

Preparing for sample analysis.

1. Samples need to be in range of 25-500ng/ul. If they are above range, the readings are not accurate and samples *must* be run again. Dilute samples with RNase free water (or DEPC water).
2. Pulse spin ladder aliquot and samples, then denature at 70C for 2 minutes and immediately put on ice. After cooled, pulse spin samples and ladder.

Bring down stairs when playing with Aaron, Jill, Jerry, and Archana.

1. PE 10 and tips, Gel Dye Mix, Ladder, Nano Marker (green top), diskette, chips, and ice bucket with samples.

Prep chip at Agilent facility:

- a. To prime machine, decontaminate electrodes.
  - i. Fill electrode chip marked ZAP with 350ul RNase ZAP and a second electrode chip marker H<sub>2</sub>O with 350ul RNase free water.
  - ii. Place ZAP chip in Agilent 2100 Bioanalyzer (with lid closed) for 1 minute.
  - iii. Subsequently insert H<sub>2</sub>O chip for 10 seconds in Agilent 2100 Bioanalyzer.
  - iv. The machine is ready for use. Repeat this procedure after each chip is analyzed. Empty electrode chips with pipette after use.
- b. To prep chip:
  - i. Take new RNA chip out of packaging and place on Chip Priming Station.
  - ii. Place 9ul of gel-dye mix in bottom of well marked with a closed circled-‘G’.
  - iii. Make sure plunger is at 1 ml, then close Chip Priming Station until ‘click’ is heard.
  - iv. Slowly press plunger until it is held by syringe clip at 0.2 ml.
  - v. Wait for exactly 30 seconds and then release plunger with clip release mechanism. Allow the plunger to rise slowly for 30 seconds. Open Chip Priming Station. *Agilent suggests looking at back of chip for bubbles; these bubbles are extremely hard to detect.*
  - vi. Place 9ul of gel-dye mix in bottom of the other two wells marked with an ‘G’.
  - vii. Place 6ul of ladder-NanoMarker in bottom of well marked with a ladder symbol.
  - viii. Dispense 5ul of NanoMarker in bottom of each **sample well**. Subsequently add 1ul of sample into each **sample well**. If less than 12 samples are to be analyzed, add 1ul of NanoMarker to remaining sample wells.
  - ix. Place chip in the adapter of vortex mixer. Vortex for 1 minute at Set-Point.

- x. Place chip in Agilent 2100 Bioanalyzer and start run within 5 minutes.
- c. To begin run:
  - i. Select Assay from Tool bar. For BioMarker samples, run Total Eukaryote RNA NanoAssay.
  - ii. Click on Start above icon of chip to open Start dialog box.
  - iii. In first pop-up box, choose number of samples to be analyzed.
  - iv. In second pop-up box, note comments concerning sample name and information. This information can also be added after the samples start running.
  - v. Click on okay to commence run. Do not hit STOP unless you desire the entire run to be void. The chip cannot be 'restarted'.
  - vi. After run is complete, print out results and save .cld file in case questions need to be addressed at a later time.
  - vii. Remember to decontaminate electrodes after each chip (or between chips).
  - viii. If happy with chip, the 4 ul aliquots can be tossed.

Labeling the samples on the cld files

1. RNA, Pre EtOH, Dil 0x, 360 ul
2. RNA, Post EtOH, Dil 4x, 15 ul
3. cRNA, Elute 1 or 2, Dil 4x, 32 ul
4. Jurkat, cRNA, Dil 4x, 30 ul