Bioanalyzer Operation

Getting Started

1. Turn on the Bioanalyzer and let the instrument warm up for at least 15 minutes

2. All reagents in the kit must be at RT for at least 30 min before use.

3. The ladder will be in 1.5ul aliquots in freezer #4 on the 3rd shelf from top. Ladders are aliquoted and frozen when a new kit comes in. The ladders for the Small RNA kit and for the RNA 6000 Nano Kit are not interchangeable.

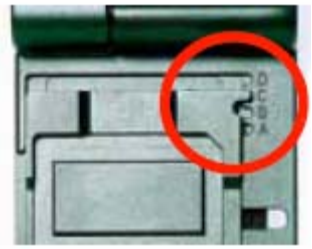
4. The RNA 6000 Nano Kit can analyze up to 12 samples and the Small RNA kit up to 11 samples.

5. Each new RNA kit comes with a replacement syringe for the chip priming station and should be replaced before using a new kit.

6. There may be aliquots of the RNA 6000 Nano gel matrix and/or Small RNA gel matrix in the walk-in refrigerator in a box next to the kits. Use these first.

7. Pipet 1.5ul of RNA samples into a 1.5ul or 0.5ul microfuge tubes (depending on what block size is in the heating block) and leave on ice until the denaturation step prior to loading the chip.

8. When using the **RNA 6000 Nano Kit** make sure the chip priming station base is in position C



and the syringe is in position the top position

9. When using the **Small RNA Kit** make sure the chip priming station base is in position C

and the syringe is at the bottom position

10. Always wear gloves, RNAse free tips, etc when handling RNA samples. Dust or other contaminants may interfere with results. Keep the dyes in kits protected from light

11. Pipet 350ul of RNAse Zap and 350ul of DEPC water into each designated electrode cleaner chip. Electrode cleaner chips are provided with each kit. They should be labeled and in a zip lock bag by the bioanalyzer.

12. Place the chip containing the RNAse Zap into the bioanalyzer, shut the lid and leave for 2 minutes.

13. Take out the chip containing the RNAse Zap and put in the chip with DEPC water and leave for 1 minute. The electrodes are now clean and ready for your samples. Repeat the same procedure after your run is complete. You can then either pipet out the RNAse Zap and DEPC water out of the electrode cleaner chips or give them a good shake to empty.

14. See instructions below for loading either a RNA 6000 Nano chip or a Small RNA Chip

RNA 6000 Nano Chip

1. RNA samples should be in the 5-500 ng/ul range. Quantify using the NanoDrop and dilute if necessary.

2. If there is not any prepared gel in the walk-in refrigerator, pipet 550ul of RNA 6000 Nano gel matrix (red top tube) into a spin filter provided in the kit.

3. Centrifuge @ 1500 g for 10 minutes at RT. Aliquot 65ul into 0.5ul microfuge tubes and store at 4C. Filtered gel is good for 4-6 weeks.

4. Vortex the RNA 6000 Nano dye concentrate (blue top tube) for 10 secs, spin down briefly and add 1ul to a 65ul aliquot of the gel matrix.

5. Vortex well and spin down for 10 min @ 13000 g at RT.

6. Put the RNA 6000 Nano chip on the priming station and add 9.0 of the gel matrix to the well marked G and close the chip priming station. **Make sure you pipet tip touches the bottom of the wells to eliminate air bubbles.**

7. Set the plunger at the 1ml mark and press the plunger down until it is held by the clip and wait exactly 30 seconds and release the clip. Wait another 5 secs and slowing pull the plunger back to the 1ml mark. Open up the chip priming station to add the rest of the reagents.

8. Pipet 9ul of gel matrix into the other two wells marked G.

9. Pipet 5ul of RNA Nano Marker (green top tube) into the well marked ladder and each sample well that will be used. Pipet 6ul of RNA Nano Marker into samples wells that will not be used.

10. Put the RNA samples and ladder (1.5ul) into a 70C heating block for 2 minutes to denature the RNA. You can now load 1ul of the ladder and samples to the appropriate wells.

11. Place the chip in the vortex mixer for 1 min at 2400 RPM. **The chip must be run within 5 minutes.**

12. Click on the 2100 Expert icon to open up the software. Place chip in the bioanalyzer and select total RNA Nano Series II from the Assay drop down menu under RNA. Pick either prokaryote or eukaryote.

13. The chip will be recognized when placed in the bioanalyzer and as soon as all the Start Run Checklists are green, the start button will be initialized so you can click on it to start your run.

14. Note: you can choose to only run wells that have sample which will shorten the run time if you only have a few samples. Be sure to clean the electrodes after use.

Small RNA Chip

1. Total RNA samples should be in the 1-100 ng/ul range; small RNA (≤ 150nt) between 1-29ng/ul; and 100-1000ng/ul for oligonucleotides. Quantify using the NanoDrop and dilute if necessary.

2. If there is not any prepared gel in the walk-in refrigerator, pipet 650ul of Small RNA gel matrix (red top tube) into a spin filter provided in the kit.

3. Centrifuge @ 10000 g for 15 minutes at RT. Aliquot 40ul into 0.5 microfuge tubes and store at 4C. Filtered gel is good for 4-6 weeks. **Gel matrix is very viscous, pipet slowly to avoid air bubbles especially when loading the chip.**

4. Vortex the RNA 6000 Nan dye concentrate (blue top tube) for 10 secs, spin down briefly and add 2ul to a 40ul aliquot of the gel matrix.

5. Mix well by pipeting or flipping over and flicking the tube and spin down for 10 min @ 13000 g at RT.

6. Put the Small RNA chip on the priming station and add 9.0 of the gel matrix to the well marked G and close the chip priming station. **Make sure you pipet tip touches the bottom of the wells to eliminate air bubbles. Pipet slowly.**

7. Set the plunger at the 1ml mark and press the plunger down until it is held by the clip and wait exactly 60 seconds and release the clip. (Remember the syringe is at the bottom position for the Small RNA Kit) Look to see if the plunger moves back to the 0.3 ml mark. Wait another 5 secs and slowing pull the plunger back to the 1ml mark. Open up the chip priming station to add the rest of the reagents.

8. Pipet 9ul of gel matrix into the other two wells marked G.

9. Slowly pipet 9ul of the Small RNA Conditioning Solution (white top tube) to the well marked CS.

10. Pipet 5ul of RNA Nano Marker (green top tube) into the well marked ladder and each sample well that will be used. Pipet 6ul of RNA Nano Marker into samples wells that will not be used.

11. Put RNA samples and ladder (1.5ul) into a 70C heating block for 2 minutes to denature the RNA. You can now load 1ul of the ladder and samples to the appropriate wells.

12. Place the chip in the vortex mixer for 1 min at 2400 RPM. **The chip must be run within 5 minutes.**

13. Click on the 2100 Expert icon to open up the software. Place chip in the bioanalyzer and select total Small RNA from the Assay drop down menu under RNA.

14. The chip will be recognized when placed in the bioanalyzer and as soon as all the Start Run Checklists are green, the start button will be initialized so you can click on it to start your run.

15. Note: you can choose to only run wells that have sample which will shorten the run time if you only have a few samples. Be sure to clean the electrodes after use.