

Agilent Bioanalyser RNA 6000 Pico Assay

(50-5000pg/ μ L)

1. Allow RNA Pico reagents need to equilibrate to room temperature for **30 minutes** before use.
2. Cleaning the electrodes:
 - **do not use RNase ZAP for decontaminating the electrodes prior to using them with a Pico kit. Please use the RNase ZAP free electrode head. Please ask a member of the genomics core to change for you.**
 - Take electrode cleaner labelled water and fill one of the wells with 350 μ L of RNase free dH₂O.
 - Place electrode cleaner in Bioanalyser, close lid and leave it for **5 minutes**.
 - Open lid and remove electrode cleaner, and leave Bioanalyser lid open for **30 seconds** to allow the water on the electrodes to evaporate.
 - Close the Bioanalyser lid.
3. If necessary prepare filtered gel matrix:
 - a. Pipette 550 μ l of RNA gel matrix (**red**) into top of spin filter provided.
 - b. Spin at 1500g for **10 minutes** (DO NOT spin using rpm setting)
 - c. Remove spin filter. Aliquot 65 μ l filtered gel into **amber** microcentrifuge tubes (one aliquot can be used for 2 chips on the same day).
 - d. Label with date. Store at 4°C in the green plastic box and use within **one month**.
4. Prepare gel-dye mix:
 - a. Vortex RNA dye concentrate (**blue**) for **10 seconds** and spin down briefly.
 - b. To 65 μ l filtered gel aliquot add 1 μ l of the RNA dye concentrate
 - c. Vortex thoroughly. Check that proper mixing of gel and dye has occurred.
 - d. Spin tube for **10 minutes** at room temperature at 13 000g.
 - e. Keep at 4° C and protected from light until needed. Use within one day and discard unused gel-dye mix.
5. Concentration of RNA should be between 200-5000pg/ μ l. If necessary dilute samples with RNase-free water. The MaC recommends a concentration of 2500pg/ μ L.
6. Thaw an aliquot of the diluted RNA 6000 Ladder (from -80°C freezer) on ice. If you use the last one, please let Genomics Lab staff know.
7. Denature RNA samples by incubating at 70°C for 2 minutes. **DO NOT HEAT THE DILUTED LADDER.**
8. Place denatured samples on ice.
9. Check that the Chip Priming Station O ring is not dirty. If it is, contact a member of the Genomics Lab, who will clean and if necessary replace it. The Chip Priming Station must be set at base plate **position C** and the syringe clip at the **topmost** position. Place RNA 6000 Pico chip in the Chip Priming Station.
10. Pipette 9 μ l of gel-dye mix into bottom of well marked .

11. Ensure syringe plunger is at 1ml. Close the Chip Priming Station and push down plunger until it is held by the syringe clip.
12. Wait for **30 seconds exactly** and then release plunger with the clip release mechanism. Allow the plunger to move back to the 1ml position, aid if necessary.
13. Pipette 9µl of gel-dye mix into the 2 remaining wells marked **G**.
14. Pipette 9µl of RNA 6000 Pico conditioning solution (white) into the well marked CS (bottom right).
15. Pipette 5µl of RNA 6000 Pico Marker (**green**) into the 12 remaining empty wells (including that marked ).
16. Pipette 1µl of diluted RNA 6000 Ladder into the well marked .
17. Pipette 1µl of each sample into its assigned well. If running fewer than 11 samples, an extra 1µl of the RNA 6000 Pico Marker should be added in place of the sample.
18. Place the chip in adapter of vortex mixer. Check that chip is secure and vortex speed is that of the 'set' point. Vortex the chip for **1 minute exactly**. DO NOT vortex for longer as this will lead to poor results.
19. To run the chip, place in Bioanalyser. It fits one way and must not be forced. Once prepared chips must be used within **5 minutes** because reagents can evaporate.
20. Close the lid. If there are problems, check the chip is inserted properly. DO NOT force the lid as this will damage the electrodes.
21. If the chip has been detected it will appear in the top left hand corner of the software screen.
22. From the "Assay" icon select 'RNA', then for total RNA select 'Eukaryote Total RNA Pico Series II' and for cRNA select 'Eukaryotic mRNA Pico Series II'.
23. Name your samples in the chart (optional), and adjust the 'Run Sample' value to the number of samples you are running.
24. Click on the 'Start' icon.
25. In the Start dialog box assign a file name.
26. The assay run takes approximately 30 minutes. DO NOT touch the Bioanalyser during an assay run. Movements of the Bioanalyser during a run may result in erroneous results.
27. Once finished remove the chip and perform the electrode cleaning H2O electrode cleaner for 1minute and lid open for **10 seconds**, before running further chips or shutting down. Empty electrode cleaners by flicking out and then gently tapping upside down onto a paper towel.