**Total RNA Quantification with Agilent RNA 6000 Nano Kit**

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When opening a new kit, perform the following steps under Kit Preparation.

**Kit Preparation**

1. Replace the syringe with the one that comes with the kit:
   1. Remove the old syringe from the lid by unscrewing and release from the clip.
   2. Remove the plastic cap of the new syringe and insert it into the clip.
   3. Slide the syringe into the hole of the Luer-Lok adapter and screw tightly.
2. Adjust the base plate:
   1. Open the priming station.
   2. Using a screwdriver, open the screw on the bottom of the base plate.
   3. Lift the base plate and insert it again at position C. Retighten the screw.
3. Adjust the syringe clip:
   1. Release the lever of the clip and slide it up to the top position.
4. Prepare the RNA Ladder:
   1. Spin ladder down and pipette into a 2.0 mL tube.
   2. Heat denature for 2 min at 70 ºC. Transfer to ice immediately.
   3. Prepare 5.0 µL aliquots in 0.5 mL tubes as needed for daily use.
   4. Store aliquots of denatured ladder at -80 ºC until needed.

**Reagent Preparation**

1. Prepare the gel:
   1. Pipette 550 µL of RNA gel matrix into a spin filter.
   2. Centrifuge at 1,500 x g for 10 min at room temperature.
   3. Aliquot 65 µL of filtered gel into 0.5 mL tubes. Store at 4 ºC and use filtered gel within 4 weeks.
2. Prepare the gel-dye mix:
   1. Allow RNA dye concentrate to equilibrate to room temperature for 30 min in darkness.
   2. Vortex for 10 sec and spin down briefly.
   3. Add 1.0 µL of dye into a 65 µL aliquot of filtered gel.
   4. Vortex solution well. Spin tube at 13,000 x g for 10 min at room temperature.
   5. Use prepared gel-dye mix within one day.

**Electrode Cleaning**

1. Make sure the electrode cartridge dedicated for RNA is installed.
2. Clean the electrode each time before running a chip.
3. Designate one of the electrode cleaning chips to RNaseZAP and one to RNase-free water.
4. Fill one of the wells of the RNaseZAP chip with 350 µL RNaseZAP.
5. Place the chip into the Bioanalyzer, close the lid, and leave for 1 min.
6. Remove the chip and keep for future use.
7. Repeat the steps with the other cleaning chip using RNase-free water.

**Chip Loading**

1. Put a new chip on the priming station.
2. Load the gel-dye mix:
   1. Pipette 9.0 µL of gel-dye mix into well 12.
   2. Make sure the plunger is positioned at 1.0 mL and then close the lid.
   3. Press plunger until it is held by the clip.
   4. Wait for exactly 30 sec, then release clip.
   5. Wait for 5 sec, then slowly pull plunger back to 1.0 mL position.
   6. Open the priming station and pipette 9.0 µL of gel-dye mix into wells 4 and 8.
3. Load the marker:
   1. Pipette 5.0 µL of RNA marker in all 12 sample wells and in the ladder well (16).
4. Load the ladder and samples:
   1. Pipette 1.0 µL of prepared ladder in the ladder well (16).
   2. Pipette 1.0 µL of sample in each of the 12 sample wells. Pipette 1.0 µL of RNA Marker in each unused sample well.
   3. Put the chip in the IKA vortexer and vortex for 1 min at 2,400 rpm.
   4. Run the chip in the Bioanalyzer within 5 min.
5. Load the chip into the Bioanalyzer and run:
   1. Start the Agilent 2100 Expert software before loading the chip.
   2. Under Instrument, select the RNA assay under the Assay menu.
   3. Accept or modify the File Prefix as needed.
   4. Enter sample IDs into the sample name table.
   5. Select the Start button; the run should take 30 min.
   6. Remove the chip when complete.
   7. Repeat the Electrode Cleaning steps above.