BioAnalyzer Protocol

For use with the Agilent High Sensitivity DNA Kit for BioAnalyzer.

Note: Dye and dye mixtures are light sensitive and decompose when exposed to light. Store these reagents in a dark place, and remove light covers only when pipetting.

BioAnalyzer reagents are also temperature sensitive and should be refrigerated at 4°C when not in use. Reagents should be allowed to come to room temperature before use in the protocol.

Note: Please refer to the BioAnalyzer Quick Start Guide or detailed Quantification Protocol for assistance with setting up the chip priming station including replacing the syringe and adjusting the base plate.

Equipment:
→ Agilent 2100 Bioanalyzer System
→ Chip priming station
→ IKA vortex mixer
→ Microcentrifuge (>13000g)
→ P10, P20, P200 pipettes

Consumables:
→ 1.5mL low-bind microcentrifuge tubes
→ Pipette tips: P20, P200

Reagents:
→ Agilent High Sensitivity DNA Kit

Preparing the Gel-Dye Mix

This step only needs to be completed if you have run out of gel-dye mix, or if it has been more than 6 weeks since you last prepared the mixture.

Protocol:

1. Allow High Sensitivity DNA dye concentrate (blue) and High Sensitivity DNA gel matrix (red) to equilibrate to room temperature for 30 min.
2. Add 15 µL of High Sensitivity DNA dye concentrate (blue) to a High Sensitivity DNA gel matrix vial (red).
3. Vortex solution well and spin down. Transfer to spin filter.
4. Centrifuge at 2240g (+/- 20%) for 15 minutes. Protect solution from light. Label the tube with the date prepared and store at 4°C when not in use. Use prepared gel-dye mix within 6 weeks of preparation.

Loading the Gel-Dye Mix

Protocol:

1. Allow the gel-dye mix to equilibrate to room temperature for 30 min before use.
2. Put a new High Sensitivity DNA chip on the chip priming station.
3. Pipette 9µL of gel-dye mix into the well marked G.
4. Make sure that the plunger is positioned at 1mL and then close the chip priming station.
5. Press the plunger until it is held by the clip.
6. Wait for *exactly* 60 seconds then release the clip.
7. Wait for 5 seconds, then slowly pull back the plunger to the 1mL position.
8. Open the chip priming stations and pipette 9µL of gel-dye mix in the wells marked G.

Loading the Marker, Ladder, and Samples

Protocol:

1. Pipette 5µL of marker (green) in all sample and ladder wells. Do not leave any wells empty.
2. Pipette 1µL of High Sensitivity DNA ladder (yellow) into the well marked with the ladder symbol.
3. In each of the 11 sample wells, pipette 1µL of sample (used wells) or 1µL of marker (unused wells).

   Note: This kit is optimized for DNA concentrations of 100pg/µL to 10ng/µL. If you suspect your libraries have concentrations of greater than 10ng/µL, dilute samples prior to loading on BioAnalyzer.
4. Put the chip horizontally in the adapter and vortex for 1 min at the indicated setting (2400 rpm).
5. Run the chip in the Agilent 2100 Bioanalyzer instrument within 5 min.

Starting an Agilent 2100 Bioanalyzer Run

Protocol:

1. Open the lid of the Agilent 2100 Bioanalyzer and carefully insert the chip. The chip fits only one way.
2. Carefully close the lid and ensure that the 2100 Expert software screen (**Instrument** tab) shows that you have inserted the chip into the machine.
3. On the **Instrument** tab, select the appropriate assay (dsDNA) from the Assay menu.
4. Update the File Prefix to reflect your sequencing run, batch name, and run date.
5. Select the appropriate number of samples and enter sample information in the sample name table below.
6. Click the **Start** button in the upper right of the window to start the chip run. The incoming raw signals are displayed in the **Instrument** tab.
7. After the chip run, *immediately* remove the chip from the BioAnalyzer and dispose of it. Chips should not be left in the machine for longer than 1 hour.

Cleaning Electrodes after a BioAnalyzer Run

Clean the BioAnalyzer electrodes shortly after each run. Cleaning should proceed promptly after each run to minimize contamination.

Protocol:

1. Slowly fill one of the wells of the electrode cleaner with 350µL deionized analysis-grade water. Make sure you use a new electrode cleaner each time you open a new BioAnalyzer kit.

   Note: More thorough cleaning is required between runs if you use the BioAnalyzer for other assays.
2. Open the lid and place the electrode cleaner in the BioAnalyzer, just as you would place a chip.
3. Close the lids and leave it closed for about 10 seconds.
4. Open the lid and remove the electrode cleaner.
5. Wait another 10 seconds to allow the water on the electrodes to evaporate before closing the lid.