Agilent 2100 Bioanalyzer (PSC 533)

Every time you use the bioanalyzer, you MUST sign the logbook.
Always wear gloves.
See below for Agilent tips and order information.

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RNA 6000 Nano Assay for RNA

1. Bring reagents to RT for 30 min.
2. Gel-Dye mix
    a. Place 400 µl of RNA gel matrix (red dot; part of kit) into the top receptacle of a spin filter (included in kit).
    b. Place the spin filter in a microcentrifuge and spin at 4000 rpm (1500 g) for 10 min. Use filtered gel within 4 weeks.
    c. Place 130 µl of the filtered RNA gel matrix into an RNAse free 1.5 ml microfuge tube and add 2 µl RNA dye concentrate (blue dot).
    d. Cap the tube. Vortex thoroughly and visually inspect proper mixing of gel and dye. Protect from light. Before use, have dye concentrate at RT. Store at 4°C. Use within 1 week.
3. Prepare sample and marker.
    a. dilute sample 1:10 and 1:100. Put 2 µl RNA ladder in tube.
    b. Heat the tubes 70°C for 2 min. Place on ice immediately.
4. Decontaminate the electrodes
    a. Slowly fill one of the wells of an electrode cleaner chip (comes with kit) with 350 µl RNAse Zap.
    b. Put in Agilent 2100 Bioanalyzer for 1 min. Lift lid and place chip in scanner. Close lid.
    c. Slowly fill one of the wells of another electrode cleaner chip with 350 µl RNAse-free water.
    d. Put in Agilent 2100 Bioanalyzer for about 10 sec.
    e. Open the lid and remove the electrode cleaner.
    f. Wait another 10 sec. For water to evaporate.
5. Loading the Gel-Dye mix – this is the hard part, if you don't get the gel distributed evenly and without bubbles throughout the channels, you won't get good results. You are supposed to be able to look at the back of the chip to see bubbles, but I could never see anything.
    a. Take a new RNA chip out of it's sealed bag.
    b. Place the chip on the chip priming station.
    c. Draw up 9.0 µl of the gel-dye mix with a pipette.
    d. Place the tip of the pipette at the bottom of the well marked with a Black circled "G" (right, second from bottom, above ladder symbol) and dispense the gel-dye mix.
    e. Make sure that the plunger is at 1 ml. Then close the chip priming station.
    f. Press the plunger until it is held by the syringe clip.

g. Wait for exactly 30 sec. Slip plunger off clip and wait 5 sec., then very slowly pull the plunger back to the 1 ml mark. It almost moves up on it's own. Open Chip Priming Station
h. Check for bubbles.
i. Pipette 9 μl of the gel-dye mix in each of the wells marked with an uncircled "G" (right top 2 wells).

6. Loading the RNA 600 Nano Marker
    a. Draw up 5 μl of the RNA 6000 Nano Marker (green dot).
    b. Place in the well marked with the ladder symbol.
    c. Dispense 5 μl of the RNA 6000 Nano Marker into each of the 12 sample wells.
    d. Draw up 1 μl RNA 6000 ladder and put it in the well with the ladder symbol.
    e. Pipette 1 μl of each sample and put in each sample well. Pipette 1 μl of RNA Nano Marker in each unused well.
    f. Put in special chip vortexer for 1 min. Run sample within 5 min.
    g. Place the chip in the Agilent 2100 Bioanalyzer scanner. You can only put the chip in 1 way. Close door.
7. Run
    a. Open Agilent 2100 Biosizing icon on desktop
    b. Graphic of scanner (upper left hand corner of window) shows when door is open or closed and also shows when a chip is in place.
    c. Click on assay
    d. Select RNA/select type of RNA in expt. Eukaryote Total RNA
    e. Click Start button/start
    f. Name your file (date, user expt. Name). Designate where you want the file stored.
    g. Fill in sample sheet
    h. At the bottom left of the window, you can see the status of the run. It will say Warning for ~ 5 min., then Calibrate, Leading, reading ladder and finally reading sample. After about 30 sec. You can start to see the sample on the window.
8. Results
    a. Select how you want to view results as they are coming off. View/single wells. Or View/ all wells or View/View Gel. Select by buttons or single wells can be selected from all well view by double clicking on the well you want to look at.
    b. Usually as the samples are coming off, view all wells.
    c. When viewing single wells, you see additional information. The left panel has: Fragment #, name, start time, end time, are, % of total area. The right panel has tabs: sample (this has the information you entered on the sample sheet as you started the run), settings (minimum and maximum ht for a called peak), RNA (start and end time for different RNA's such at 18S etc.), Results (RNA area, RNA concentration and tRNA ratio, 28S/18S) and Error (let's you know if any peaks are missing in the ladder).
    d. Right click to get additional choices such as "scale to selected" or "scale all"
    e. Print results.

Protein 200 Plus Assay

1. Bring reagents to RT for 30 min.
2. Gel-Dye mix
    a. Place 25 µl of protein dye concentrate (blue dot) to Protein gel matrix tube (red dot; part of kit). Vortex solution well and transfer into the top receptacle of a spin filter (included in kit).
    b. Place the spin filter in a microcentrifuge and spin at 4000 rpm (2500 g) for 15 min. Use filtered gel within 4 weeks.
3. Destaining solution
    a. Place 650 µl of the gel matrix (red dot) into spin filter.
    b. Place the spin filter in a microcentrifuge and spin at 4000 rpm (2500 g) for 15 min. One tube is sufficient for 1 kit (25 chips).
4. Prepare Denatured soln.
    a. Remove 1 vial of sample buffer from freezer. Allow to warm to RT and then vortex well.
    b. For reducing conditions: Add 7 µl of b-mercaptoethanol or 7 µl of dithiothreitol to 200 µl sample buffer. For non-reducing conditions: add 7 µl of deionized water to 200 µl sample buffer.
    c. Vortex for 5 seconds.
5. Decontaminate the electrodes
    a. Slowly fill one of the wells of an electrode cleaner chip (comes with kit) with 350 µl RNAse Zap.
    b. Put in Agilent 2100 Bioanalyzer for 1 min. Lift lid and place chip in scanner. Close lid.
    c. Slowly fill one of the wells of another electrode cleaner chip with 350 µl RNAse-free water.
    d. Put in Agilent 2100 Bioanalyzer for about 10 sec.
    e. Open the lid and remove the electrode cleaner.
    f. Wait another 10 sec. for water to evaporate.
6. Preparing the samples and the Ladder
    a. Combine 4 µl of protein sample and 2 µl denaturing soln. In a 0.5 microfuge tube. Mix well and spin down for 15 sec.
    b. Pipette 6 µl of ladder in a 0.5 microfuge tube.
    c. Place each sample tube and the ladder tube for 3 – 5 min. at 95 – 100 ^{o}C or in boiling water.
    d. Spin down each tube 15 sec.
    e. To each sample and ladder tube add 84 µl of deionized water and vortex.
7. Loading the Gel-Dye mix – this is the hard part, if you don't get the gel distributed evenly and without bubbles throughout the channels, you won't get good results. You are supposed to be able to look at the back of the chip to see bubbles, but I could never see anything.
    a. Take a new protein chip out of it's sealed bag.
    b. Place the chip on the chip priming station.
    c. Draw up 12.0 µl of the gel-dye mix with a pipette.

d. Place the tip of the pipette at the bottom of the well marked with a black circled "G" (right, top) and dispense the gel-dye mix.
e. Make sure that the plunger is at 1 ml. Then close the chip priming station.
f. Press the plunger until it is held by the syringe clip.
g. Wait for exactly 60 sec. And then release the plunger with the clip release mechanism.
h. Remove solution in well G.
i. Pipette 12 µl of gel-dye mix in the circled G well and the uncircled G well (right second and third well from top and bottom row next to ladder well).
j. Pipette 12 µl of destain solution in well labeled DS (bottom right well)
k. Open the chip priming station and check for bubbles.

8. Loading the samples and the ladder
    a. Pipette 6 µl of sample in 10 sample wells.
    b. Dispense 6 µl of the ladder into the well marked with the ladder symbol (bottom, second well from the left)
    c. Place the chip in the Agilent 2100 Bioanalyzer scanner. Close door.
9. Run
    a. Open Agilent 2100 Biosizing icon on desktop
    b. Graphic of scanner (upper left hand corner of window) shows when door is open or closed and also shows when a chip is in place.
    c. Click on assay
    d. Select Protein/select 200 Plus
    e. Click Start button/start
    f. Name your file (date, user expt. Name). Designate where you want the file stored.
    g. Fill in sample sheet
    h. At the bottom left of the window, you can see the status of the run. It will say Warning for ~ 5 min., then Calibrate, Leading, reading ladder and finally reading sample. After about 30 sec. You can start to see the sample on the window.
10. Results
    a. Select how you want to view results as they are coming off. View/single wells. Or View/ all wells or View/View Gel
    b. Right click to "scale to selected" or "scale all"
    c. Print results.

DNA 500/1000 Assay

1. Bring reagents to RT for 30 min.
2. Gel-Dye mix
    a. Vortex the DNS dye concentrate (blue dot) 10 sec. and spin down. Make sure the DMSO is completely dissolved.
    b. Place 25 µl of DNA dye concentrate (blue dot) to DNA gel matrix tube (red dot; part of kit). Store the dye concentrate at 4°C. Cap the tube, vortex 10 sec. Visually inspect proper mixing of gel and dye.
    c. Transfer into the top receptacle of a spin filter (included in kit).

d. Place the spin filter in a microcentrifuge and spin at 6000 rpm (2240 g) for 15 min. Label the tube with the date. Use filtered gel within 4 weeks. Protect from light and store at 4°C.

3. Loading the Gel-Dye mix – this is the hard part, if you don't get the gel distributed evenly and without bubbles throughout the channels, you won't get good results. You are supposed to be able to look at the back of the chip to see bubbles, but I could never see anything.
    a. Take a new DNA chip out of it's sealed bag.
    b. Place the chip on the chip priming station.
    c. Draw up 9.0 μl of the gel-dye mix.
    d. Place the tip of the pipette at the bottom of the well marked with a black circled "G" (right, top) and dispense the gel-dye mix.
    e. Make sure that the plunger is at 1 ml. Then close the chip priming station. You should hear a click when the Priming Station is closed correctly.
    f. Press the plunger until it is held by the syringe clip.
    g. Wait for exactly 60 sec. Slip plunger off clip and wait 5 sec., then very slowly pull the plunger back to the 1 ml mark. It almost moves up on it's own. Open Chip Priming Station.
    h. Pipette 9 μl of the gel-dye mix in each of the wells marked "G" (no black circle).
    i. Pipette 5.0 μl of the gel-dye mix in the well marked with the ladder symbol.
4. Loading the Marker, samples and the Ladder
    a. Dispense 6 μl of the Marker (green dot) into each of the 12 sample wells. Add 6 μl of the Marker to wells even if unused.
    b. Pipette 1 μl of the DNA ladder (yellow dot) in the well marked with the ladder symbol.
    c. Pipette 1 μl of sample in each of the 12 sample wells.
    b. Place the chip in the adapter of the vortex mixer. Vortex for 60 sec.
    c. Place the chip in the Agilent 2100 Bioanalyzer scanner. The chip only fits 1 way. Close door.
    d. Be sure to start the run within 5 min.
5. Run
    a. Open Agilent 2100 Biosizing icon on desktop.
    b. Graphic of scanner (upper left hand corner of window) shows when door is open or closed and also shows when a chip is in place.
    c. Click on assay
    d. Select DNA 500 or DNA 1000
    e. Click Start button/start
    f. Name your file (date, user expt. Name). Designate where you want the file stored.
    g. Fill in sample sheet
    h. At the bottom left of the window, you can see the status of the run. It will say Warning for ~ 5 min., then Calibrate, Leading, reading ladder and finally reading sample. After about 30 sec. You can start to see the sample on the window.

6. Results
    a. Select how you want to view results as they are coming off. View/single wells. Or View/ all wells or View/View Gel. Select by buttons or single wells can be selected from all well view by double clicking on the well you want to look at.
    b. Usually as the samples are coming off, view all wells.
    c. When viewing single wells, you see additional information. The left panel has: Peak, Mig Time, Corr area, size (bp), conc. (ng/µl), Molarity. The right panel has tabs: sample (this has the information you entered on the sample sheet as you started the run), settings (minimum and maximum ht for a called peak), Results (DNA area, DNA concentration), and Error (let's you know if any peaks are missing in the ladder).
    d. Right click to get additional choices such as "scale to selected" or "scale all"
    e. Print results.

Bioanalyzer tips (from Agilent)

1. Electrode cleaning. All assays are sensitive to voltage variations caused by residue on the electrodes. Results can be erratic due to dirty electrode pins.
    a. Remove chips promptly after each run and use the cleaning chip
    b. Clean monthly or sooner if the electrode is suspected to be dirty.
    c. For models G2938B and C, remove the electrode from the cassette and soak 15 min. in DI water or RNAse free (if RNA assays are employed).
    d. The electrode is not removable from the cassette on model A instruments so remove the cassette & clean as well as possible without getting the cassette wet.
    e. Sonicate or use a soft toothbrush gently
    f. RNAse ZAP (for RNA assay users).
    g. Rinse well; final rinse with 100% EtOH; dry 2 hrs.
2. Prime properly. While being a robust platform, priming issues can crop up even with seasoned users.
    a. We recommend having a Priming station maintenance kit on hand (G2938-68716).
    b. Change the syringe with each new kit.
    c. Make sure that all users follow the priming sequence verbatim. If anyone alters it, they may get some backflow into the station orifice, which will likely give erratic assay results.
    d. Inspect the white O-ring on the bottom of the station orifice. Clean or replace as needed.
    e. If the orifice may be dirty, use an old syringe to run clean water and then EtOH through the orifice and into the sink. Let dry.
3. Prep the chip
    a. Let reagents warm up to room temperature, vortex, and give a quick microfuge spin. Get all reagents and samples ready before priming.
    b. Protect the dye and gel-dye mix from light.

c. Generally 2000 rpm is enough and avoids some issues of flicking liquid out of the wells. If your vortexer top is worn, an improved one is available (part 5022-2190).
d. Have the chip loaded and running within 5 to 10 minutes of priming.

5. Infreq. Users. You may make smaller batches of gel-dye mix, but would need extra Corning Spin-X 0.22 um filter tubes (our part 5185-5990). If your reagents expire, you may purchase reagents without chips for about half the cost.
6. Avoid contamination. To avoid dust and other confounding contaminants: wear powder free gloves, don't handle the bottom or top of the chips or place on dusty surfaces. Close the BioA lid when not in use and/or clean the lens with lens paper and 70% isopropanol.
7. Exploit the software. Expert software has many features and a very extensive help menu. Click through the toolbar menu options and the Setpoint Explorer.
8. Explore the possible. Agilent is continually coming up with assays for the Bioanalyzer and users perfect their own independently or in collaboration with Agilent. We maintain a listing of published paper available upon request. Also consult our web library at http://www.chem.agilent.com/Scripts/PCol.asp?lPage=50
9. Tech Support. When you need us, call Tech Support at 1-800-227-9770 (options 2,2,2)

Maintenance and Troubleshooting

1. Chip not detected. The gaskets and o-rings in the priming station need to be replaced. Apparently if the priming station isn't working properly, the liquid level in the chip isn't right, and the chip isn't detected.

Part #'s: Syringe adaptor replacement kit - #G2938-68716

(a) Syringe for priming station – G2938-68706

Ordering

1.Rnase Zap – Ambion, cat. #: 9780.9782
2. RNA 6000 Pico Labchip kit – Agilent, cat. #: 5065-4473; $540.00; there is also a Nano kit. See below for website.
3. DNA 500 LabChip kit - Agilent, cat. #: 5064-8284; $331.00; there is also a 1000, 7500 and 12000 bp kit. See below for website.
4. Protein 50 LabChip Kit - Agilent, cat. #: 5065-4484; $524.00; there is also a 200 kDa kit; see below for website
5. For additional pricing and catalog information, see Bioanalyzer Catalog