Table of Contents

1.0 Purpose

The purpose of this Procedure is to describe the general use and maintenance of Agilent 2100 Bioanalyzer.

2.0 Scope

This SOP applies to Biopharmaceutical Development Program (BDP)/ QC personnel operating the Agilent Bioanalyzer 2100 Systems.

3.0 Authority and Responsibility

This section defines the personnel, supervisors, and/or departments and their individual responsibilities.

3.1 The Director, Process Analytics/ Quality control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to with Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for following general lab safety (power/electrical, heat/ flammable material, UV radiation) hazard and regulatory precautions during the performance of this procedure.

3.4 PA/QC personnel are responsible for generating/reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Equipment, Materials

4.1 Agilent 2100 Bioanalyzer

4.2 Isopropanol, Reagent -Grade, BDP PN 30882 or BDP approved equivalent

4.3 Kimwipes, Lint -Free Lens Wipe, BDP PN 20091 or BDP approved equivalent

4.4 Direct-Q-Water, or BDP approved equivalent

4.5 RNaseZAP, BDP PN 22649 or BDP approved equivalent

4.6 Soft brush (toothbrush)

4.7 Oil-free compressed air

4.8 Syringe KIT, BDP PN 22647 or BDP approved equivalent

4.9 Screwdriver or equivalent

4.10 Gasket kit, BDP PN 22648 or BDP approved equivalent

4.11 Agilent protein 230 kit, BDP PN 31395 or BDP approved equivalent

5.0 Procedure

5.1 Check the Chip Priming Station for proper performance (seal test)

5.1.1 Replace the syringe with each new reagent kit (after 25 chip runs). Unscrew the old syringe from the lid of Chip Priming station.

5.1.2 Release the old syringe from the clip. Discard the old syringe.

5.1.3 Remove the plastic cap of the new syringe and insert it into the clip.

5.1.4 Slide it into the hole of the luer lock adaptor and screw it tightly to the Chip Priming Station.

5.1.5 Ensure the syringe is tightly connected to the Chip Priming Station.

5.1.6 Pull the plunge of the syringe to the 1.0 mL position

5.1.7 Place an unused chip in the Chip Priming Station.

5.1.8 Close the Chip Priming Station. The lock of the latch will audibly click when it closes.

5.1.9 Press the plunger down until it is locked by the clip.

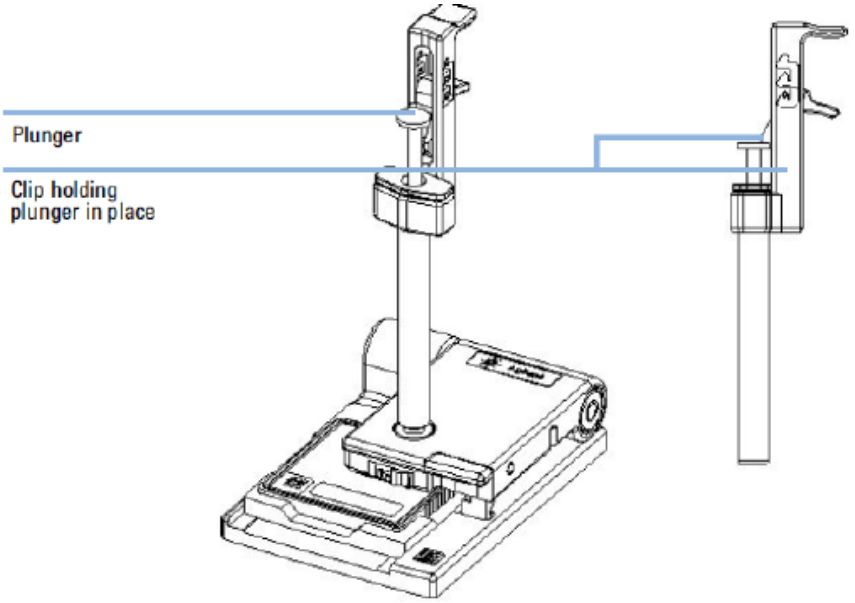


Figure 8 Locking the plunger of the syringe with the clip

5.1.10 Wait for 1 minute and lower latch of the clip to release the plunger as shown in the following image.

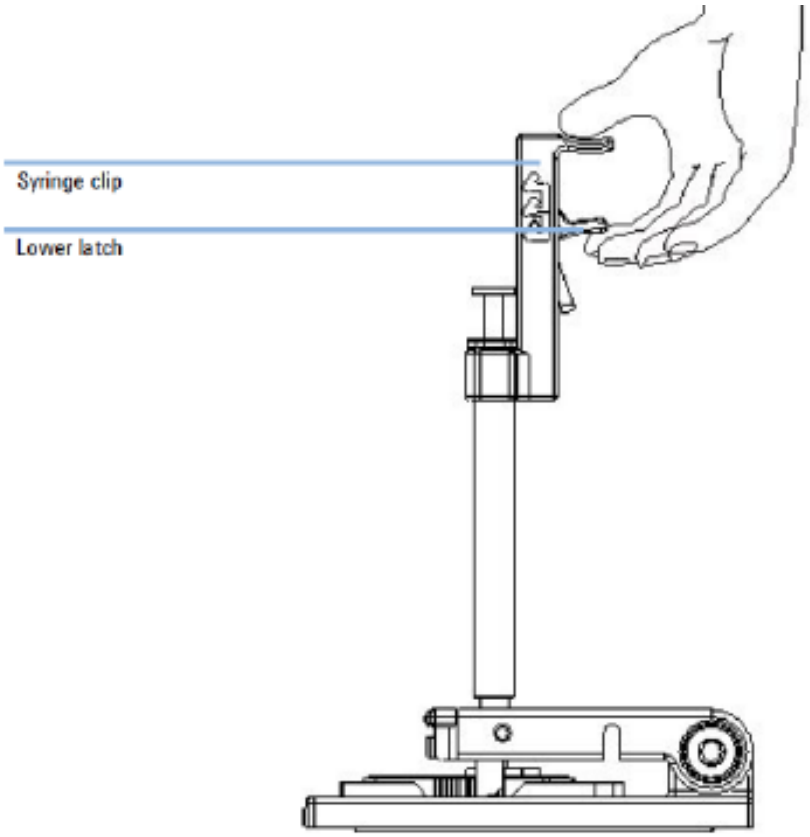


Figure 9 Releasing the plunger from the syringe

5.1.11 To indicate an appropriate sealing, the plunger should move back up at least to the 0.3mL mark within less than 1 second.

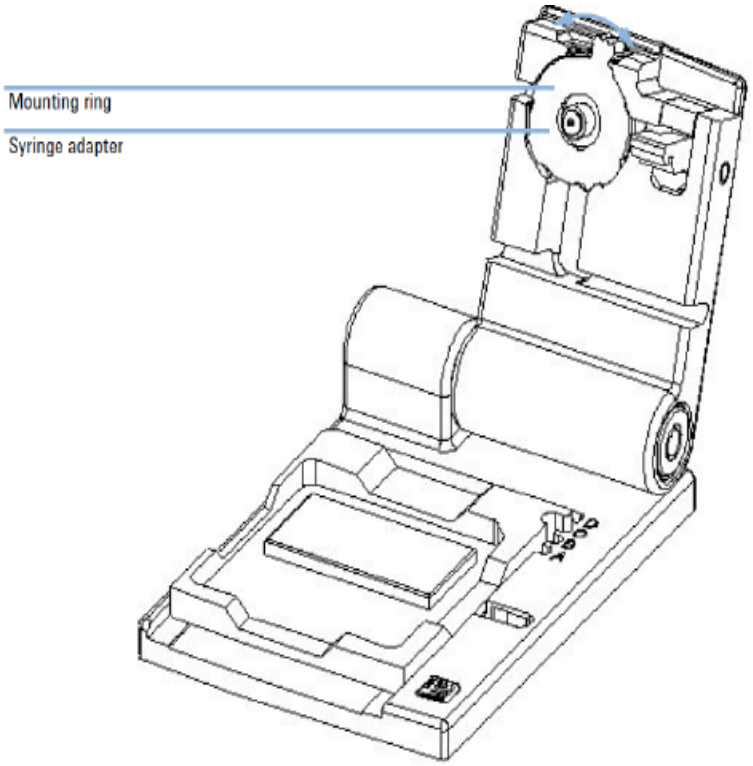
5.1.12 If the lunger does not move up to the 0.3 mL mark within a second, the syringe-chip connection is probably not tight enough. Retighten the syringe or replace the syringe adaptor, syringe or gasket to fix the problem.

5.1.13 Repeat Step 5.1.6 to 5.1.11 for proper performance of a seal test. If the prime station still does not move up to the 0.3 mL mark within a second, replace the syringe adaptor and gasket according to step 5.2.

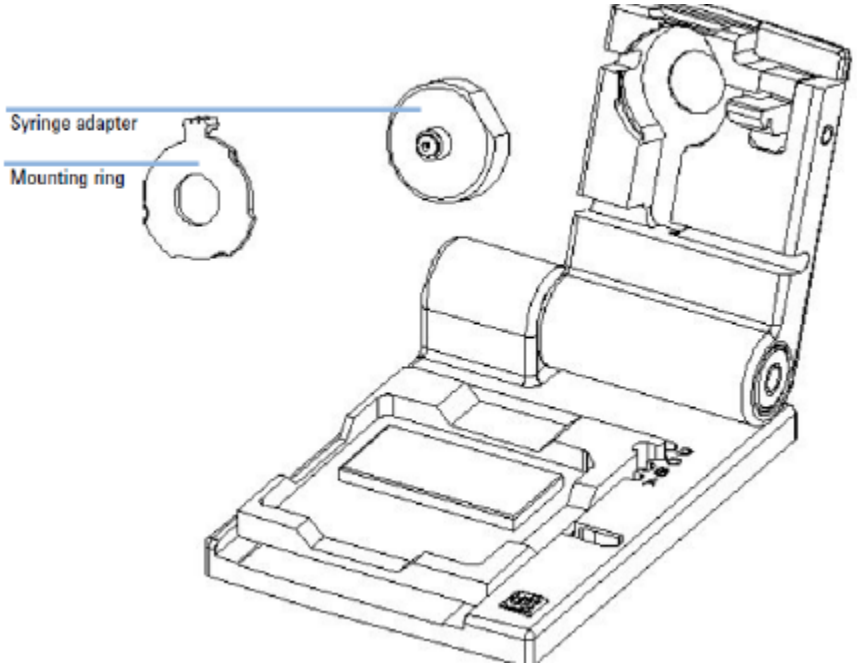
5.2 Clean and replacing the syringe adapter and gasket

5.2.1 Open the priming station.

5.2.2 Move the mounting ring holding the adaptor in place to the left as shown below. The ring will come off.



5.2.3 Press the syringe adaptor out of its mount as shown below.



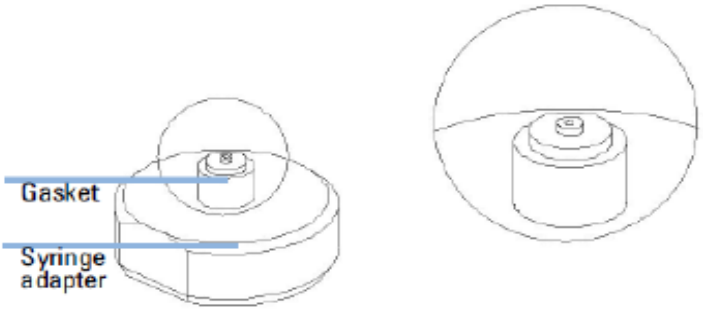
5.2.4 Removed dried gel at the opening of the adaptor with a needle.

5.2.5 If cleaning adaptor, screw on syringe and flush water through the adaptor several times.

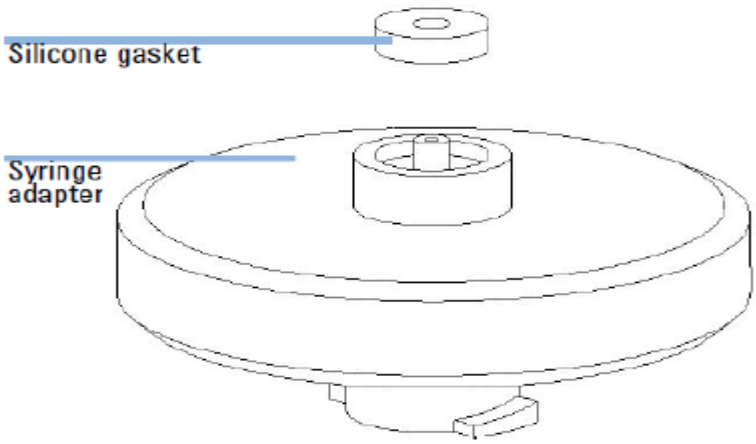
5.2.6 If cleaning syringe adaptor, flush syringe with isopropanol.

5.2.7 Allow adaptor to dry fully.

5.2.8 Insert the cleaned syringe adaptor or replace syringe adaptor and gasket



5.2.9 Pull out the old silicone gasket with fingers or forceps.



5.2.10 Assemble the priming station.

5.2.11 Close the Chip Priming Station.

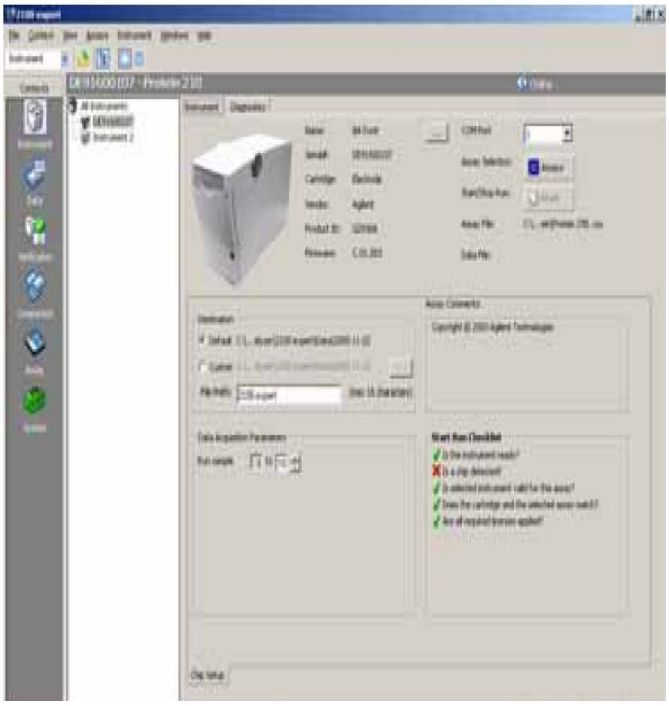
5.2.12 Screw a dry syringe tight into the luer lock adaptor.

5.2.13 Perform a seal test described in step 5.1.

5.3 Start Chip Run

5.3.1 Ensure the Agilent Bioanalyzer is connected to line power and connected to the computer. Turn on the line at the rear panel. The status indicator at the front of the Agilent 2100 Bioanalyzer comes on and shows green.

5.3.2 Double-click the 2100 Expert Software to start. The screen of the software appears in the Instrument context. The icon in the upper part of the screen represents the current instrument communication status.



Lid closed, no chip or chip empty

Lid open

Dimmed icon: no communication

Lid closed, chip inserted, protein or demo assay selected

5.3.3 Click the Assays menu then select an assay for the chip run.

5.3.4 Prepare the samples and the chip according to SOP 23001.

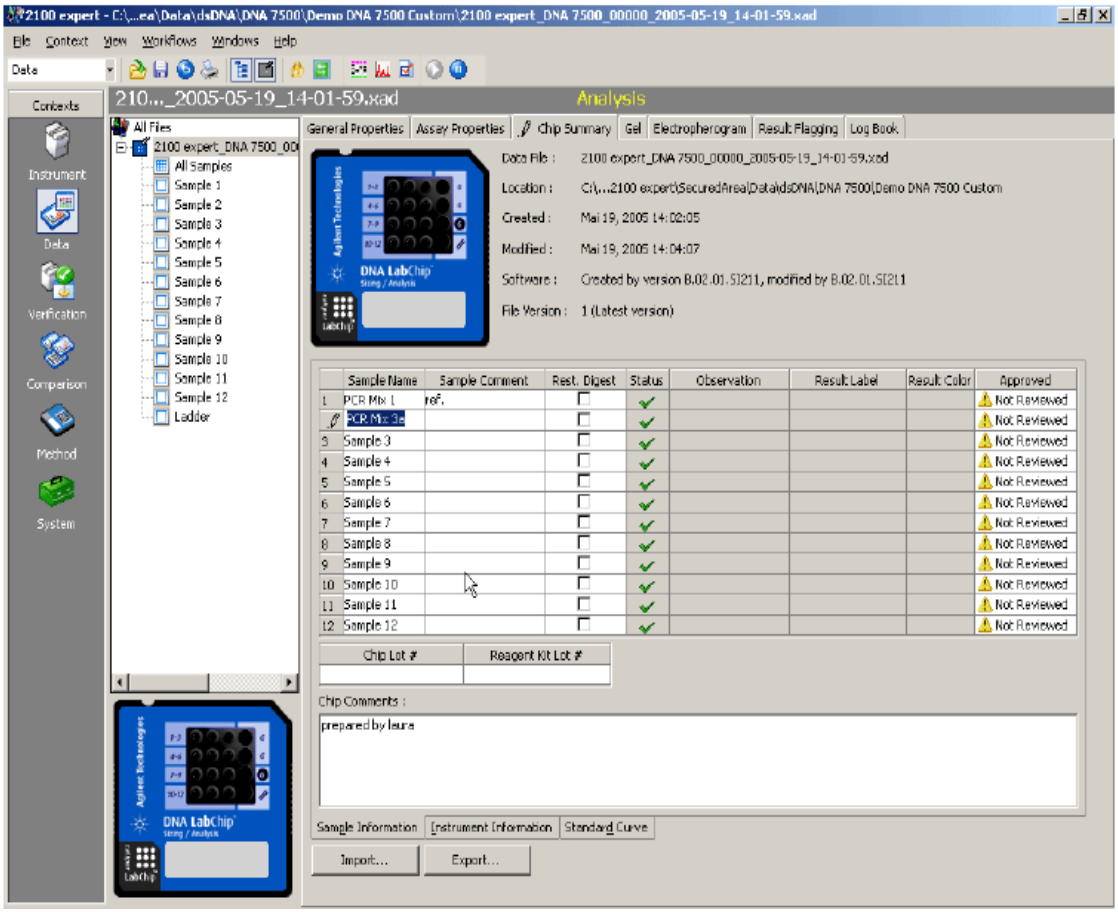
5.3.5 Place the chip into receptacle. Carefully close the lid. When the chip is detected, the image on the Instrument tab changes to a chip. Click Start in the Upper right of the window.

5.3.6 Once run has finished remove chip from Bioanalyzer. Save data to the PA/QC Scientific Data, “Bioanalyzer 2100-Agilent” Save the data by using the QC test request number or the date assay was performed. For example QC-073534 030323.

5.3.7 Clean Electrodes per step 5.5**.**

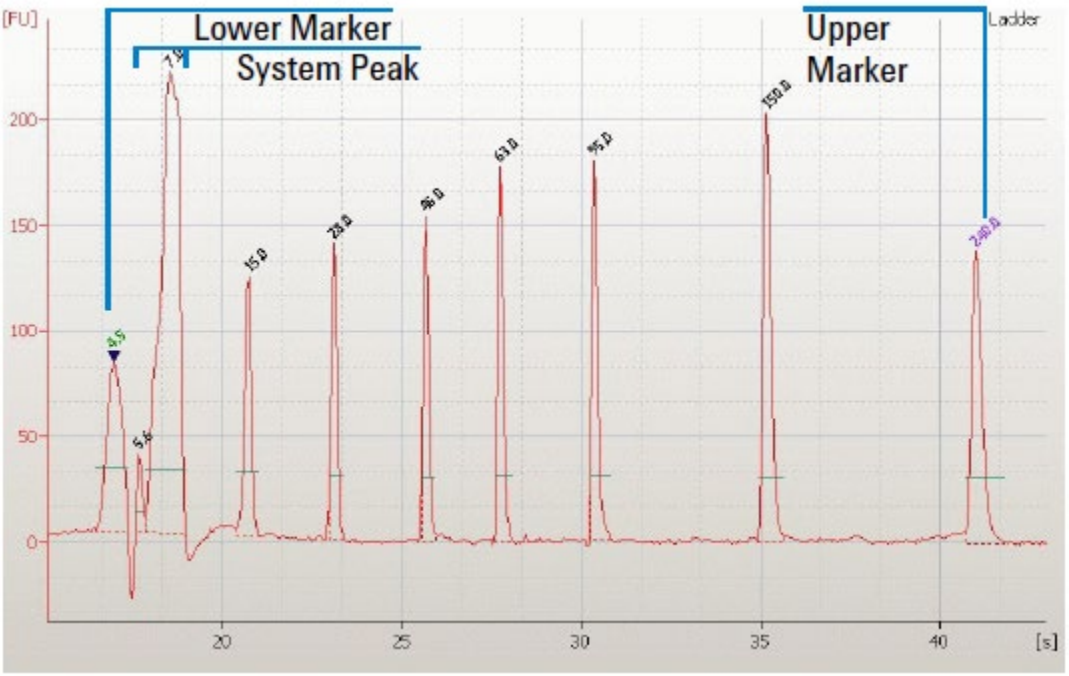
5.4 Evaluating Electrophoretic data (2100 Expert Software)

5.4.1 To view the results, switch to the Data Context. The Chip Summary tab shows information on your chip data file.



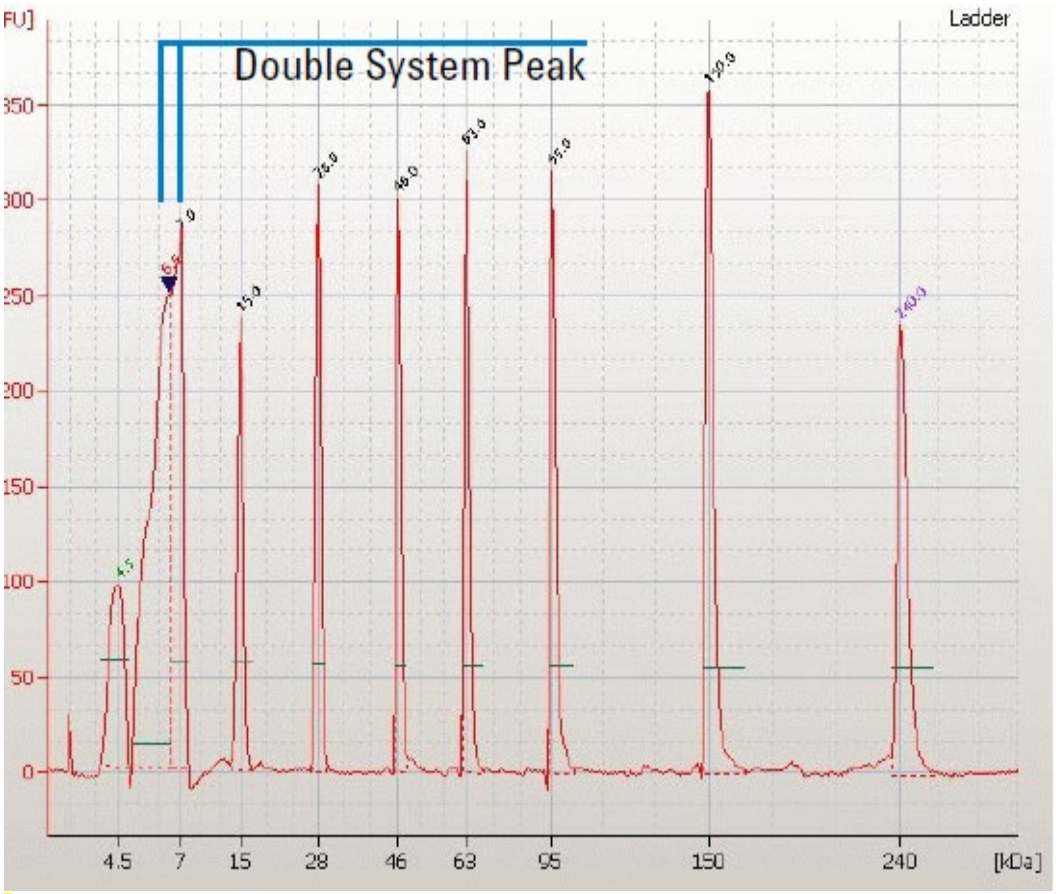
5.4.2 In the tree View panel, click any sample name or the ladder. Under Electropherogram tab, peak have automatically been detected, and their characteristics such as size, concentration, purity or molarity have been calculated. A sizing ladder is run first from the ladder wall. The size of the individuall protein are present as kDa in the assay. The concentrations of each peak may vary slightly from ladder to ladder.

5.4.3 To check the Ladder results, select the electropherogram tab in the Data context. The electropherogram of the protein 230 ladder should resemble as shown below.

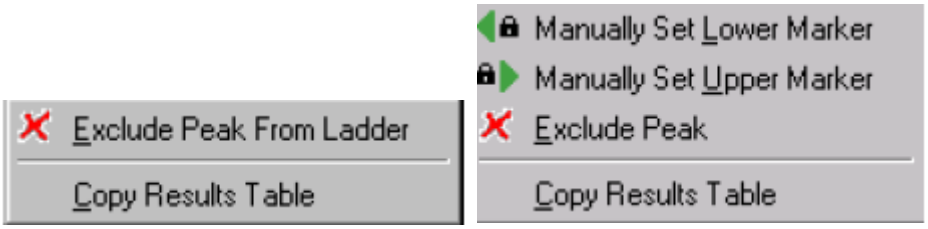


5.4.4 Major features of a successful ladder run are 7 ladder peaks in which all peaks are resolved and have a flat baseline .

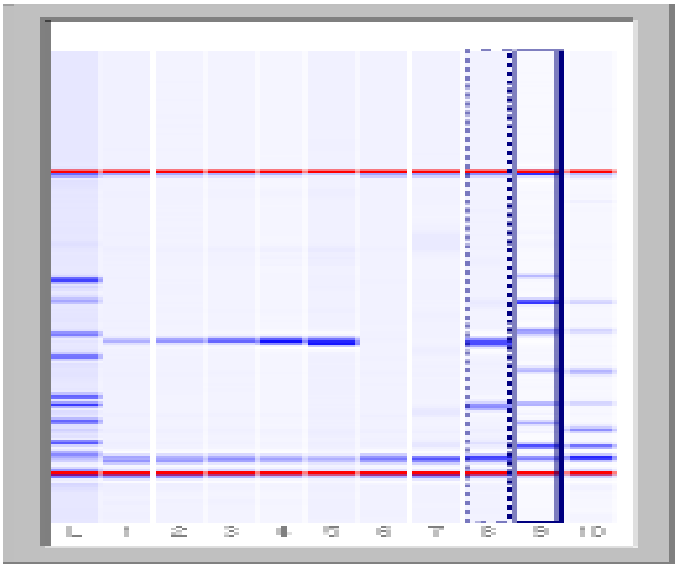
5.4.5 In case both system peaks are identified as ladder peaks, exclude peak 2 (the left of the two peaks by moving the cursor over the second peak and click the right mouse button. Select exclude peaks from ladder to make the change.



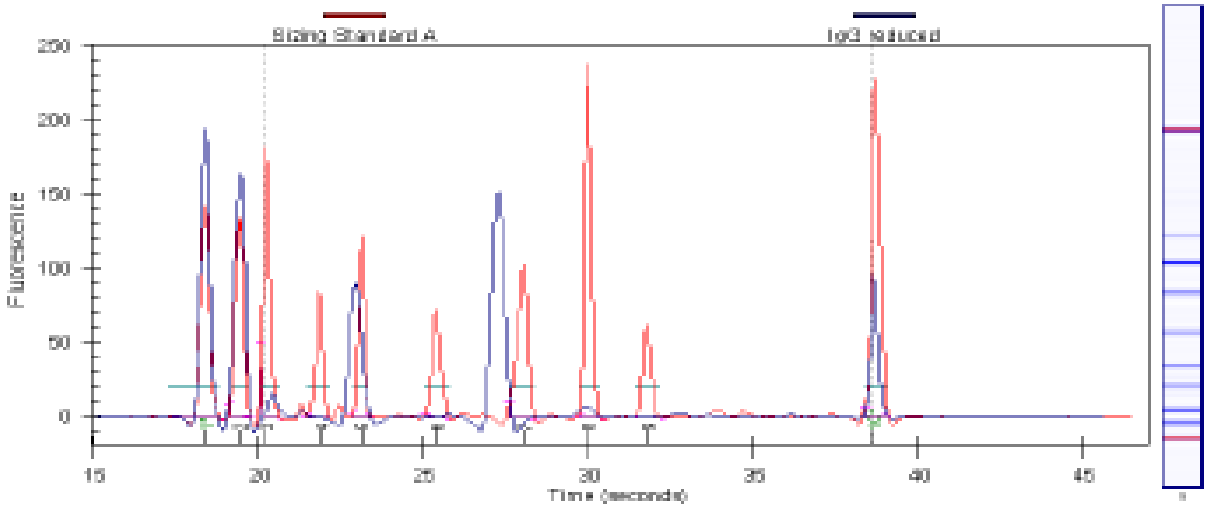
.



5.4.6 Overlaying electropherograms: Data from multiple wells can be overlaid within the single well large display menu view. Hold down the CTRL key and then click the left mouse buttons or other lanes in the gel image in the lower left corner of the screen. Remove wells from the overlay by CTRL + clicking the corresponding lane in the small gel display (the bounding box will disappear).



Sample 9 with sample 8 overlaid



5.5 Clean Electrodes Daily During Use

5.5.1 Fill Electrode Cleaner (a clean chip included in kit) with 350 µL of Deionized water for protein 230 chip or RNaseZap for RNA 6000 Chip.

NOTE: After 25 Chip runs, the contents of a chip kit, replace the Electrode Cleaner with an Electrode Cleaner from a new chip kit.

Never fill more than 350 µL of water in the electrode cleaner. Liquid spill may cause currents between the electrodes.

5.5.2 Open Bioanalyzer lid and seat Electrode cleaner.

5.5.3 Close lid and leave it closed for approximately 10 seconds for protein 230 chips or 1 minute for RNA 6000 chips.

5.5.4 Open lid and remove Electrode Cleaner.

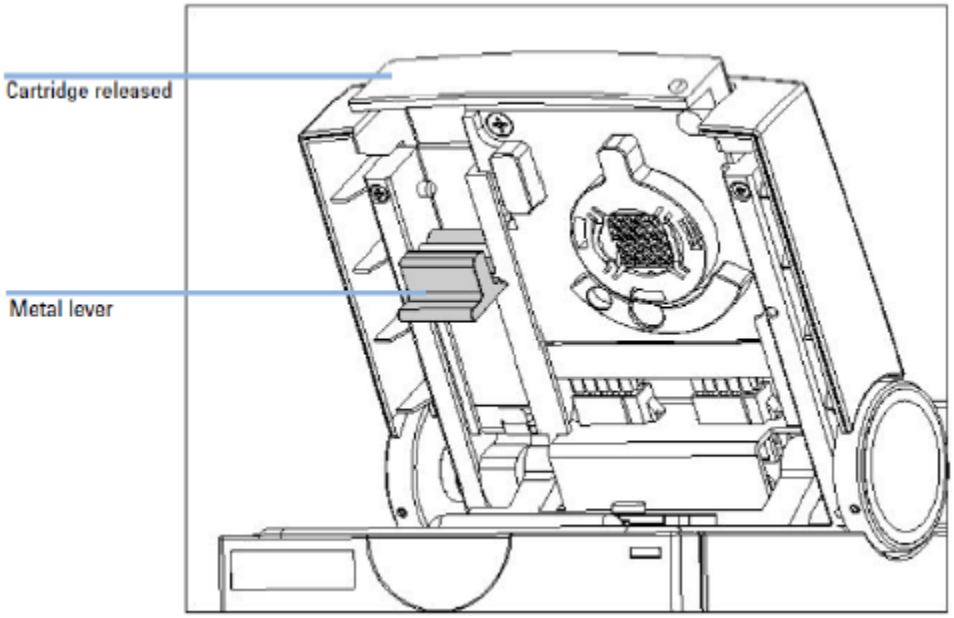
5.5.5 Wait for approximately 10 seconds for electrodes to dry before closing the lid.

5.5.6 Empty the electrode cleaner after cleaning procedure and refill the electrode cleaner.

5.6 Clean the Pin Set (yearly)

5.6.1 Turn off power to the 2100 Bioanalyzer instrument.

5.6.2 Open the lid and pull the metal lever on the inside left of the lid to vertical position as shown in the following image. When the lever is in the vertical position, the cartridge is released from the lid by about 10 mm.



5.6.3 Gently pull the cartridge out of the lid.

5.6.4 Open the bayonet socket of the pin set by turning the plastic lever to the left as shown below.

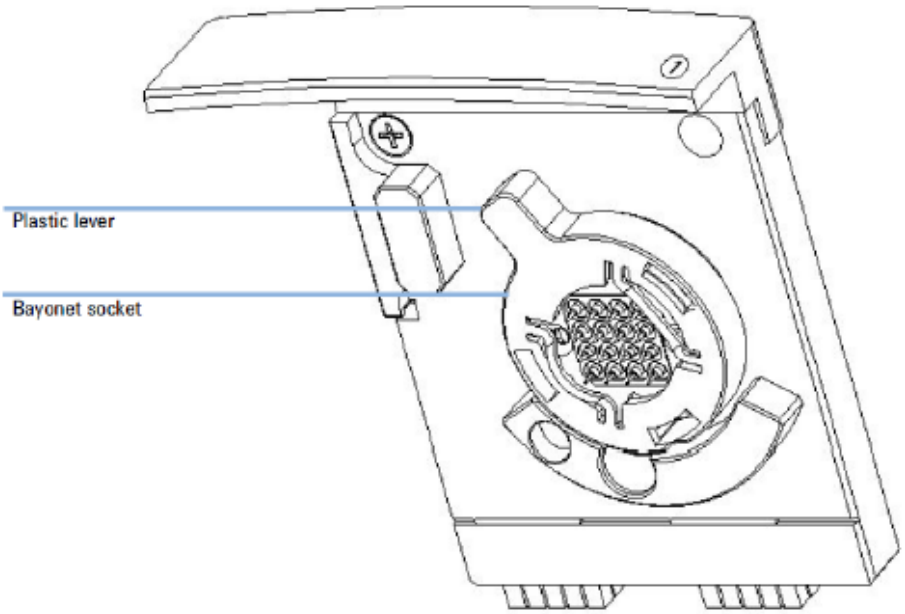
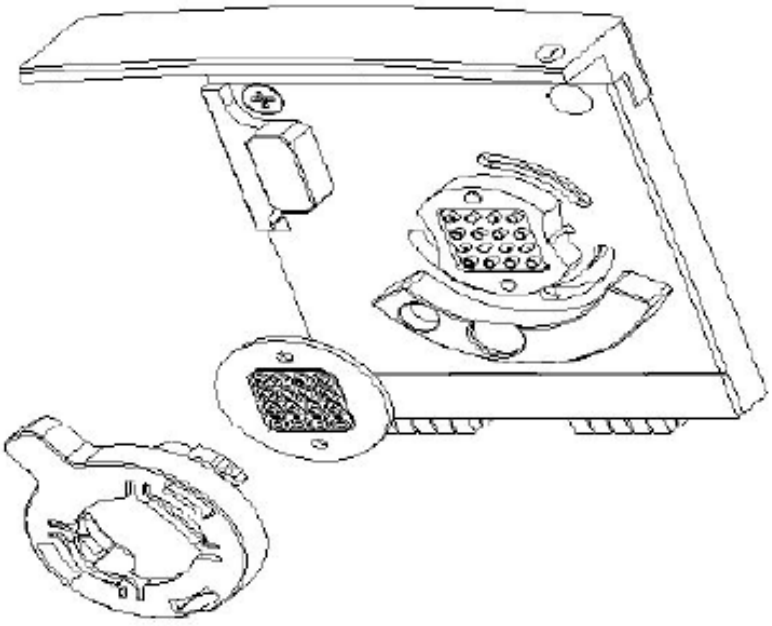


Figure 2 Bayonet socket of the electrode pin set

5.6.5 Remove the cover of the bayonet socket by gently pulling the plastic lever. The pin set may stick to the electrode base. Remove it by carefully pulling it off.



5.6.6 With Soft brush gently clean pin set with RNase Zap or isopropanol when using Bioanalyzer for DNA and protein Assays.

NOTE: Bending or misaligning the pins will lead to poor quality results or prematurely terminated assay runs.

5.6.7 Let the pin set completely dry in a desiccator overnight or use oil-free compressed air.

NOTE: Ensure that the pin set is fully dry before placing back into the electrode base. Even small amounts of liquid on the pin set can damage the high voltage power supply.

5.6.8 Place the pin set on the cartridge base and the bayonet cover over the pin set.

5.6.9 Lock the pin set to the electrode base by turning the plastic lever of the bayonet cover to the right.

5.6.10 Slide the electrode cartridge with the pin set into the 2100 Bioanalyzer instrument lid and move the metal lever to the flat (closed) position.

5.6.11 Push the metal front of electrode cartridge to ensure a tight connection to the 2100 Bioanalyzer instrument.

5.6.12 To verify that the electrodes are completely dry, perform the short circuit diagnostic test from the Diagnostics tab in the instrument context. This test takes approximately three minutes.

NOTE: Heat can permanently damage the electrode cartridge. Do not dry the electrode cartridge in an oven.

5.6.13 If the short circuit test fails, the electrode assembly may still be wet. Take the pin set out of the instrument, dry it again with oil-free compressed air, then repeat the test.

NOTE: The limits of the short circuit test specify an ambient temperature of 25°C and relative humidity less than or equal to 50%. Higher temperatures and relative humidity could result in a leak current. Using a fresh chip is required.

5.6.14 Record cleaning in the equipment logbook.

5.7 Lens cleaning (quarterly cleaning)

5.7.1 To clean the lens, switch the instrument off.

5.7.2 Open the lid of the instrument and wipe with a lint-free lens wipe dampened with reagent-grade isopropanol.

5.7.3 Gently swab the surface of the lens.

5.7.4 Allow the alcohol to evaporate prior to closing lid or use.

NOTE: Liquid dripping into the instrument could cause a shock or damage the instrument.

5.7.5 Record cleaning in the equipment logbook.

6.0 References and Related Documents

Agilent 2100 Bioanalyzer Use Manual

Agilent 2100 Exert Software Install User Manual

SOP 23001 *Protein Analysis with the Agilent 2100 Bioanalyzer System*

SOP 21531 *Equipment Logs*