
Experion™ Pro260 Analysis Kit Instruction Manual



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Section 1

Introduction

1.1 Background Information

LabChip microfluidic technology provides new opportunities in the development of proteomic and genomic expression analysis. The Experion automated electrophoresis system (Figure 1.1) employs LabChip microfluidic technology to automate electrophoresis for protein and RNA analysis. This microfluidic-based system integrates separation, detection, and data analysis within a single platform. Refer to Appendix D in the Experion Automated Electrophoresis Station Instruction Manual for additional information on how the Experion system employs this technology.

At the center of the Experion system is the microfluidic chip, which contains a series of plastic wells bonded over a small glass plate. The glass plate is etched with an optimized network of microchannels, one of which intersects with each of the plastic wells. Once these channels have been primed with a gel matrix and the samples applied to the appropriate wells, the electrophoresis station directs the samples through these microchannels by specifically controlling the voltages and currents that are applied. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software are designed to

accomplish separation, staining, destaining, detection, and basic data analysis without any user intervention.



Fig. 1.1. The Experion system includes the following components: (1) Automated electrophoresis station, (2) Priming station, (3) Vortex station for RNA analysis, (4) System operation and data analysis tools, and (5) Analysis kits, which include the (a) chips and (b) reagents for protein (Pro260 kit), standard-sensitivity RNA (StdSens kit), and high-sensitivity RNA analysis (HighSens kit).

Using the reagents and supplies included with the Pro260 analysis kit, the Experion system can achieve separation and analysis of proteins from 10 kD to approximately 260 kD. The Experion Pro260 analysis kit uses precision-engineered lower and upper internal alignment markers to provide clean baselines, and the most accurate microfluidic-based molecular weight sizing and protein quantitation analysis.

1.2 Product Description

Each Experion analysis kit contains the chips and reagents required to perform protein electrophoresis. Kits are available in 10- and 25-chip configurations. The Experion Pro260 analysis kit can separate and quantitate protein samples ranging from 10 to 260 kD in mass. The sensitivity of this kit is comparable to colloidal Coomassie Blue staining of SDS-PAGE gels. The Pro260 kit includes the Experion Pro260 ladder, a modified version of the Precision Plus Protein™ standards that has been optimized for automated electrophoresis on the Experion system. The Pro260 ladder contains nine highly purified recombinant proteins with molecular masses from 10 to 260 kD. The Pro260 sample buffer contains a lower marker and an upper marker that are used for the proper alignment of samples to the Pro260 ladder. The upper marker is a 260 kD protein that is also used to quantitate the protein samples. Each Experion Pro260 chip can analyze between 1 and 10 samples.

Pro260 Kit Reagents

| Item | Description | Volume/Vial | 10-Chip Kit | 25-Chip Kit |
|---------------|--|-------------|-------------|-------------|
| Pro260 gel | Proprietary polymer sieving matrix | 520 μ l | 3 vials | 5 vials |
| Pro260 stain | Proprietary fluorescent dye | 45 μ l | 1 vial | 2 vials |
| Sample buffer | Contains lower and upper alignment markers | 400 μ l | 1 vial | 2 vials |
| Pro260 ladder | Precision Plus Protein standard | 60 μ l | 1 vial | 2 vials |
| Spin filters | | N/A | 3 | 5 |

Pro260 Kit Chips

| Item | 10-Chip Kit | 25-Chip Kit |
|---------------|-------------|-------------|
| Pro260 chip | 10 chips | 25 chips |
| Cleaning chip | 1 chip | 1 chip |

1.3 Specifications

| Specification | Experion Pro260 Kit |
|------------------------|--|
| Number of sample wells | 10 |
| Sample volume required | 4 μ l |
| Total run time | ~30 min |
| Protein sizing range | 10–260 kD |
| Limit of detection | 2.5 ng/ μ l carbonic anhydrase in 1x PBS (10 ng total). Equivalent to Colloidal Commassie Blue G-250 stain |
| Linear Dynamic range | 2.5 ng/ μ l – 2000 ng/ μ l* |

1.4 Additional Equipment Requirements

Experion automated electrophoresis station

Experion priming station

Calibrated pipets

0.5 ml microcentrifuge tubes

Microcentrifuge

Heating block or water bath set at 95–100°C

Deionized water, 0.2 μ m filtered (ReadyPrep™ proteomic grade water, Catalog #163-2091)

β -mercaptoethanol (β ME) (catalog #161-0710)

Vortexer

* Determined using bovine carbonic anhydrase in 1x PBS buffer

1.5 Storage Conditions

| Item | Storage Conditions | Shelf Life |
|--------------------------------------|--------------------|-------------------------|
| Experion Pro260 reagents | 4°C | 9 months |
| Experion Pro260 chips | Ambient | 9 months |
| Gel-stain solution (see Section 2.3) | 4°C | 1 month from filtration |
| Gel solution (see Section 2.3) | 4°C | 1 month from filtration |

Section 2 Procedure

2.1 Procedure Overview

1. Filter gel and gel-stain solution.
2. Prepare sample buffer with reducing agent or water.
3. Prepare ladder and protein samples.
4. Prime chip with gel-stain solution.
5. Load chip (10–20 μl size narrow-bore tips only).
6. Run protein analysis.
7. Clean instrument electrodes.

2.2 Essential Practices

- Store all reagents at 4°C when not in use.
- Before using, allow all kit reagents to equilibrate to room temperature (~15–20 minutes). The Experion Pro260 stain contains DMSO and should be thawed completely before use.
- Gently vortex all kit reagents before use followed by a quick centrifugation to collect the solution at the bottom of the tubes.
- Protect the Pro260 stain, sample buffer, and gel-stain solution from light (see Section 2.3). Some reagents in these mixtures are light sensitive.
- Always wear gloves when handling the reagents and chips. Handle chips on the edges and do not touch the glass. The chips should only be removed from their packaging immediately prior to use.
- Avoid sources of dust or other contaminants when preparing samples and loading the chip. Foreign particles in reagents, samples, or in the wells of the chip will interfere with results.
- Coated or treated tubes (for example, siliconized polypropylene) should not be used in the preparation of the kit reagents or samples. Use of treated tubes may lead to artifacts during the separation.

- Dispense reagents into chips slowly. Only use 10 and 20 μl size narrow-bore tips for chip loading. Always insert the pipet tip vertically and to the bottom of the chip well when dispensing liquids. **Do not expel air at the end of the pipetting step.** This will reduce the possibility of air bubbles becoming trapped between the reagent and the microchannels at the bottom of the chip wells.
- Run a loaded chip within 5 minutes of loading. Excessive evaporation may otherwise occur, leading to poor results or a chip performance error.
- Do not open the electrophoresis station while running an analysis.
- Never place the electrophoresis station on or near a vibrating surface.

2.3 Equilibrating Kit Reagents

1. Remove at least 1 tube of the Pro260 Stain (**blue cap**), Pro260 sample buffer (**yellow cap**), and Pro260 ladder (**red cap**), as well as 2 tubes of Pro260 gel (**green cap**) from storage and equilibrate to room temperature for ~ 15 – 20 minutes. **Keep the stain and sample buffer covered at all times to avoid exposure to light.**

Note: If gel-stain solution and filtered gel were previously prepared it is not necessary to remove the Pro260 Stain (**blue cap**) and Pro260 Gel (**green cap**). Instead, remove the filtered gel stocks from storage and equilibrate as detailed above.

2. Vortex the contents of each tube and briefly centrifuge to bring the solutions to the bottom of the tubes. Make sure the DMSO in the stain is completely thawed before proceeding.

2.4 Preparing the Gel-Stain and the Filtered Gel

1. Add 20 μl Pro260 stain (**blue cap**) to a tube of 520 μl Pro260 gel (**green cap**). Vortex for 10 seconds at the highest setting and spin down in a microcentrifuge for a few seconds.
2. Cap the Pro260 stain tightly, since DMSO is highly hygroscopic, and store in the dark at 4°C.
3. Transfer the gel-stain solution (GS) to a spin-filter tube. Label and date the tube.
4. To make the filtered gel (G), pipet the contents (520 μl) of one tube Pro260 gel (**green cap**) into another spin-filter tube. Label and date the tube.
5. Centrifuge both spin-filter tubes for 5 minutes at 10,000 \times g.
6. Visually inspect the tubes to ensure that all of the gel has passed through the filters and then discard the filters.

Note:

- Use the gel-stain solution and filtered gel within 4 weeks of preparation. After 4 weeks, the filtered gel should be refiltered and can then be reused.

- Keep the gel-stain solution protected from light and store at 4°C when not in use.
- If using previously prepared gel-stain solution or filtered gel, allow at least 15 minutes for the reagents to come to room temperature before loading a chip.

2.5 Preparing the Sample Buffer

Note: Prepare sample buffer fresh daily.

A. Reducing Conditions (most cases)

For separating protein under reducing conditions the sample buffer (**yellow cap**) must be supplemented with a reducing agent.

1. For each chip to be run, transfer 30 μl of sample buffer to a microcentrifuge tube, and then add 1 μl of β -mercaptoethanol (BME). Vortex.

B. Nonreducing Conditions

When separating proteins under nonreducing conditions, 2 stocks of sample buffer, one reducing and one nonreducing must be prepared. Reducing sample buffer must always be added to the Pro260 ladder,

while the nonreducing sample buffer can be mixed with each sample being analyzed.

1. For each chip to be run, transfer 30 μ l of sample buffer (**yellow cap**) to each of two appropriately labeled microcentrifuge tubes. Add 1 μ l of β -mercaptoethanol (β ME) to one tube (reducing sample buffer for Pro260 ladder). Add 1 μ l of deionied water to the second tube (nonreducing sample buffer). Vortex both tubes.

2.6 Preparing the Samples and the Pro260 Ladder

1. In a 0.5 ml microcentrifuge tube mix 4 μ l Pro260 ladder (**red cap**) and 2 μ l of sample buffer prepared in Section 2.5. For each sample, add 2 μ l sample buffer to 4 μ l sample in a 0.5 ml microcentrifuge tube. Vortex tubes briefly and spin down in a microcentrifuge for a few seconds.

Note: When preparing a blank sample, treat it like any other sample and add 2 μ l of sample buffer to 4 μ l of the blank solution (for example, 1x PBS).

Note: Even when preparing samples for nonreducing conditions, the Pro260 ladder must still be prepared with reducing sample buffer (see 2.5B). Samples prepared with nonreducing or reducing sample buffer can be analyzed on the same chips.

2. Place the sample tubes and the Pro260 ladder in a 95–100°C heating block for 3–5 minutes.
3. Let the tubes cool down, and then spin down in a microcentrifuge for 15 seconds.
4. Add 84 µl of deionized water (0.2 µm filtered) to each tube and vortex briefly to mix. It is recommended not to use autoclaved water. Samples are ready to be loaded onto a chip.

Note: Both the diluted samples and the Pro260 ladder are stable for several hours when stored at room temperature and when protected from light.

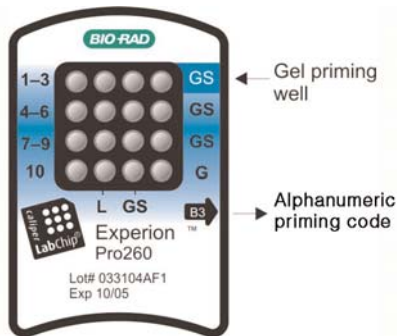
2.7 Priming the Chip

1. Open the Experion priming station by pressing down on the lever on the front.
2. Remove an Experion Pro260 chip out of its packaging and place it on the chip platform, matching the arrow on the chip with the alignment arrow on the chip platform. A post on the chip prevents insertion in the wrong position. Do not force the chip into position.

- Pipet 12 μ l filtered gel-stain solution (from Section 2.4) into the top right well labeled **GS** (gel priming well). Insert the tip of the pipet vertically and to the bottom of the well when dispensing. Dispense slowly, and do not expel air at the end of the pipeting step.

Warning: Placing the pipet tip at the edge of the well or allowing the gel to slide down the wall of the well may lead to bubble formation at the bottom of the well. It is acceptable to allow 1–2 small bubbles at the surface. Dislodge bubbles at the bottom with a clean pipet tip.

- Carefully close the priming station by gently pressing down on the lid. The lid should snap completely closed.
- Set the pressure setting to **B** and the time setting to **3**, as specified by the alphanumeric code on the chip.



6. Press the **start** button. The “Priming” message will illuminate on the LCD screen, the priming station will pressurize, and the timer will count down. Complete priming requires approximately 60 seconds. Do not open the priming station during the count-down.
7. An audible signal indicates that the priming is complete, and a “Ready” message will be displayed. Open the priming station by pressing down on the release lever.
8. Turn the chip over and inspect the microchannels for bubbles or evidence of incomplete priming. The glass chip will appear opaque and the microchannels will be difficult to see if they are primed properly. If you detect a problem, such as a bubble or incomplete priming, prime a new chip.
9. Reposition the chip on the priming station chip platform for subsequent well loading steps. Alternatively, the chip can be placed on a clean surface for loading.

2.8 Loading the Samples and Pro260 Ladder Into the Chip

1. Pipet out and discard any remaining gel-stain solution from the top right well labeled **GS** (gel priming well).
2. Pipet 12 μ l filtered gel-stain solution into all four wells labeled **GS** (including priming well).
3. Pipet 12 μ l filtered gel into the well labeled **G**.
4. Inspect the wells to make sure that there is no excessive bubble formation from pipetting. This can be done by holding the chip above a light-colored background and looking down through the wells.
5. Pipet 6 μ l diluted samples into the sample wells, 1–10.
 - Make sure the pipet tip is centered and positioned vertically all the way to the bottom of the well. Avoid introducing bubbles into the bottom of the wells. It is acceptable to allow 1–2 small bubbles at the surface. For best success use narrow-bore 10–20 μ l pipet tips.
 - Do not leave any wells empty, or the chip will not run properly. If necessary, pipet a blank sample, replicate sample, or replicate ladder into any empty sample well.



6. Pipet 6 μ l diluted Pro260 ladder into the ladder well labeled **L**. The Pro260 ladder **must** be prepared fresh and used within 8 hours. Every chip **must** have the Pro260 ladder loaded into the ladder well labeled **L**. Protect ladder preparation from light.
7. Start the run immediately. Run a loaded chip immediately (within 5 minutes) or excess evaporation may occur, leading to poor results or a chip performance error.

2.9 Running the Pro260 Analysis

1. Turn on the power to the Experion electrophoresis station by pushing the green button in the center of the front panel. The steady green LED above the button indicates that the unit is on.
2. Launch the Experion software.
3. Open the lid of the electrophoresis station. Place the primed chip, which has been loaded with samples, on the chip platform. Ensure that the chip is seated properly and then carefully close the lid.
4. Select **New Run** and **Experion Pro260** protocol.
5. Click the **Start** button to begin the chip run.
6. After a run has started, the green LED in the center of the front panel on the electrophoresis station will begin blinking.

7. When the chip run is complete, a “Run complete” message will be displayed. Remove the chip from the electrophoresis station and dispose it properly. Do not leave the chip in the electrophoresis station for an extended period of time, since this may cause contamination of the electrodes. Perform the steps in Section 2.10 immediately at the end of the chip run. Do not allow the previously run samples to dry on the electrodes.

2.10 Cleaning the Electrodes After a Run

1. Fill a cleaning chip with 800 μ l deionized water (0.2 μ m filtered). Gently tap the side of the cleaning chip to remove any trapped bubbles from the wells.
2. Open the lid of the electrophoresis station and place the cleaning chip on the chip platform.
3. Close the lid and leave it closed for about 60 seconds.
4. Open the lid and remove the cleaning chip.
5. Allow the electrodes to dry for 60 seconds.
6. Close the lid.
7. Replace the water in the cleaning chip after use to avoid contamination. For storage, remove the water from the cleaning chip and store the chip in a clean location.

Section 3

Data Analysis

Examples of electropherograms generated by the Experion software are provided in this section. For detailed instructions on using the software analysis tools, refer to the software help.

3.1 Example of a Pro260 Ladder Separation

At the end of each run, the Experion software will display an electropherogram of the Pro260 ladder, which is loaded into the ladder (L) well of the Pro260 chip. The prepared Pro260 ladder generates 11–12 peaks in the electropherogram (Figure 3.1). The software will identify the first peak as the lower alignment marker at 1.2 kD and the last peak as the upper alignment marker at 260 kD. The peaks between the lower alignment marker and the first ladder peak (10 kD) are referred to as 'system peaks'. If the protein separation was successful, all 9 peaks in the prepared Pro260 ladder (10–260 kD) should be well resolved.

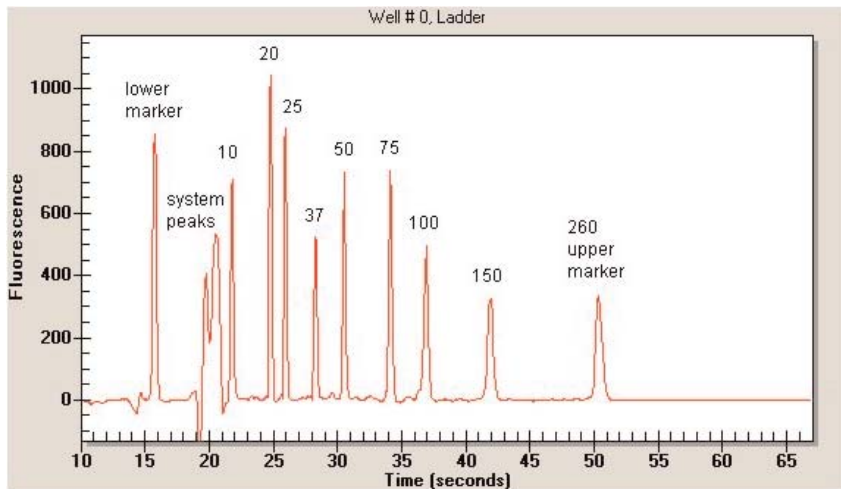


Fig. 3.1 Electropherogram of the Experion Pro260 ladder illustrating relative positions of the upper and lower markers, system peaks, and nine Pro260 ladder peaks.

3.2 Example of a Protein Sample Separation

Figure 3.2 shows an electropherogram of a sample of bovine carbonic anhydrase (100 ng/ μ l) separated with the Experion Pro260 analysis kit. The first and last peaks in the electropherogram correspond to the lower

and upper markers, which are incorporated into the Experion sample buffer and are used for alignment to the Pro260 ladder. The upper marker is also used for quantitation. The peak generated by the carbonic anhydrase sample is located between these two markers (Figure 3.2).

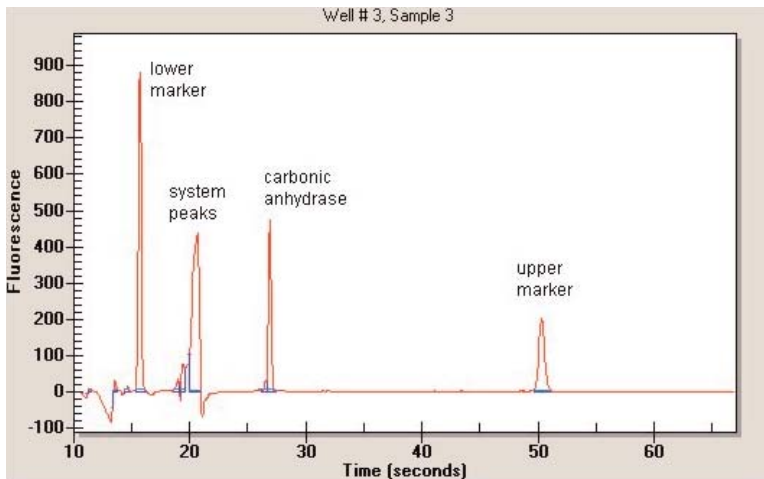


Fig. 3.2. Electropherogram of bovine carbonic anhydrase illustrating the relative positions of the lower alignment marker, system peaks, carbonic anhydrase, and upper marker.

Section 4

Chemical Compatibility List

For optimal results, the undiluted protein sample should contain components at concentrations less than or equal to the values indicated in the tables below. If concentrations are above these values, the protein samples should be diluted appropriately.

4.1 Buffers

| Buffer | Concentration |
|--------------------|---|
| IEF anode buffer | 1.5x: 10.5 mM phosphoric acid |
| IEF cathode buffer | 1.5x: 30 mM lysine, 30 mM arginine |
| Tris/Tricine/SDS | 1.5x: 150 mM Tris, 150 mM Tricine, 0.15% SDS, pH 8.3 |
| Tris/glycine/SDS | 1.5x: 37.5 mM Tris, 288 mM glycine, 0.15% SDS, pH 8.3 |
| Tris/glycine | 1.5x: 37.5 mM Tris, 288 mM glycine, pH 8.3 |
| Tris/CAPS | 1.5x: 90 mM Tris, 60 mM CAPS, pH 9.6 |
| XT MOPS buffer | 1.5x |
| XT Tricine buffer | 1.5x |
| XT sample buffer | 1.5x |

| Buffer | Concentration |
|---|--|
| Laemmli buffer | 0.5x: 31.3 mM Tris-HCl, pH 6.8, 1% SDS, 12.5% glycerol, 0.005% Bromophenol Blue, 2.5% β ME |
| Tricine sample buffer | 0.5x: 100 mM Tris-HCl, pH 6.8, 1% SDS, 20% glycerol, 0.02% Coomassie Brilliant Blue |
| Zymogram sample | 0.5x: 31.3 mM Tris-HCl, pH 6.8, 2% SDS, 12.5% glycerol, 0.005% Bromophenol Blue |
| Phosphate-buffered saline chloride, (PBS) | 1.5x: 15 mM sodium phosphate, 225 mM sodium chloride, pH 7.4 |
| Tris-borate-EDTA (TBE) | 1.5x: 133 mM Tris, 133 mM boric acid, 3 mM EDTA, pH 8.3 |
| PBS-Tween | 0.5x: 5 mM sodium phosphate, 75 mM sodium chloride, 0.05% Tween, pH 7.4 |
| IMAC elution buffer | 0.5x: 25 mM potassium phosphate, 150 mM sodium chloride, 125 mM imidazole, pH 8 |
| IMAC loading buffer | 0.5x: 25 mM potassium phosphate, 150 mM sodium chloride, pH 8 |
| Urea-phosphate-sodium chloride buffer | 0.5x: 4M urea, 25 mM potassium phosphate, 150 mM sodium chloride, pH 8 |
| Protein A - resin washing buffer I | 50 mM sodium bicarbonate, 250 mM sodium chloride, pH 8 |

| Buffer | Concentration |
|-------------------------------------|--|
| Protein A - resin washing buffer II | 50 mM sodium acetate, 250 mM sodium chloride, pH 4 |
| Bug Buster (Novagen) | 0.5x (Bug Buster and benzonase nuclease) |
| B-PER (Pierce) | 0.5x |
| Luria-Bertani (LB) medium | 0.5x: 0.5% Tryptone, 0.25% yeast extract, 0.5% sodium chloride |

4.2 Chemicals

| Chemical | Concentration | Chemical | Concentration |
|-------------------|----------------------|--------------------|----------------------|
| Acetone | 15% | Glycerol | 35% |
| Acetonitrile | 30% | | |
| Ammonium sulfate | 250 mM | HEPES | 50 mM |
| Bio-Lyte® 3/10 | 0.75% | Hydrochloric acid | 10 mM |
| Dimethylsulfoxide | 15% | Imidazole | 500 mM |
| EDTA | 150 mM | Magnesium chloride | 150 mM |
| EGTA | 150 mM | MES | 50 mM |
| Ethanol | 30% | Methanol | 15% |
| Glucose | 500 mM | MOPS | 100 mM |

| Chemical | Concentration | Chemical | Concentration |
|------------------------------|----------------------|----------------------|----------------------|
| PEG (6000) | 0.5% | Sodium citrate, pH 3 | 250 mM |
| Potassium chloride | 250 mM | Sodium hydroxide | 0.15 N |
| Sodium azide | 0.75% | Sucrose | 200 mM |
| Sodium carbonate, pH 11.6 | 150 mM | Tris base | 1.5 M |
| Sodium chloride | 250 mM | Urea | 4.5 M |

Note: High salt concentrations may decrease the sensitivity of the Pro260 analysis.

4.3 Detergents and Reductants

| Chemical | Concentration | Chemical | Concentration |
|--------------------------|----------------------|------------------|----------------------|
| ASB-14 | 0.1% | NP-40 | 0.1% |
| CHAPS | 1% | SB 3-10 | 0.1% |
| CHAPSO | 0.1% | SDS | 1.5% |
| Deoxycholate | 0.5% | TBP | 7.5 mM |
| DTT | 150 mM | Triton X-100 | 0.05% |
| LDS | 3% | Tween 20 | 0.1% |
| β -mercaptoethanol | 1.5 M | Zwittergent 3-14 | 0.1% |

Note: High concentrations of detergents may generate large system peaks that may interfere with sizing of protein samples below 20 kD.

Section 5

Troubleshooting

For more troubleshooting tips, refer to the Experion Automated Electrophoresis Station Instruction Manual.

5.1 Electrophoresis and Priming Stations

- **“IV Check Failure” message displayed on screen:**
 - One or more wells are not filled correctly and the electrodes are not completely immersed. All sample wells must be filled, using blanks or replicate samples as necessary
 - Air bubbles are interfering with the electrical contact in one or more of the wells. Tap the chip or use a clean pipet tip to remove or dislodge the bubbles. When pipetting, insert the tip vertically and to the bottom of the well. Dispense the liquid slowly. Do not expel air at the end of the pipetting step
- **“Check Seal” message lights up:**
 - Check or replace the O-ring gasket in the priming station
 - Confirm that there is a chip in the priming station and that the chip is properly positioned on the platform

- Check that the gel-stain (GS) has been added to the correct well for priming

5.2 Pro260 Analysis

- **No peaks detected in lane on electropherogram:**
 - An air bubble is in the sample well or chip due to poor priming or sample loading technique. Check the chip for air bubbles after chip priming and sample loading
 - The sample volume in well is too low. Make sure that 6 μ l solution has been added to all sample wells
 - Samples do not contain sample buffer or were not prepared properly. Review correct sample preparation procedure in Sections 2.5 and 2.6
 - Particulates are clogging channels. Use high-quality, 0.2 μ m filtered water (such as Bio-Rad ReadyPrep proteomics grade water) and verify that the gel and gel-stain solution were properly filtered. Minimize the loading of particulates in the sample by performing a quick microcentrifuge spin of the prepared sample to pellet particulates

- **Small peaks in lane on electropherogram:**
 - An air bubble is in the sample well. Check the chip for air bubbles after sample loading
 - Ensure that the chip is seated properly on the electrophoresis station chip platform
 - Pipetting error. Ensure pipets are calibrated. Use pipets that accurately deliver volumes below or equal to 10 μ l
 - One or more components in the sample is beyond the range of concentration listed in the compatibility table. High salt can decrease height of sample peaks
 - Gel-stain was incorrectly added to well labeled G
- **Poor overall chip performance:**
 - There is an air bubble in the chip. Check the chip for air bubbles after chip priming
 - Gel-stain was loaded into the wrong well during priming. Before priming, check to ensure correct loading of gel-stain solution (GS)
 - Priming station “Replace Seal” indicator light is on, but the chip was still used. Check or replace the O-ring

- Cold reagents were used. Ensure that kit reagents are warmed to room temperature before use and mixed properly
- Ensure that the reagents have not expired and light-sensitive reagents have been protected from light
- Clean the electrode with Experion electrode cleaner solution to remove any contaminants
 1. Place a cleaning chip filled with 800 μ l Experion electrode cleaner solution into the electrophoresis station
 2. Close the lid for 2 minutes. Remove the cleaning chip
 3. Place another cleaning chip filled with 800 μ l water into the electrophoresis station
 4. Close the lid for 5 minutes. Replace the water in the cleaning chip and repeat the rinse step. Remove the cleaning chip

- **Poor ladder and sample separation:**
 - The Pro260 ladder and samples were not completely denatured. Make sure the samples and the ladder were heated for 3–5 minutes at 95–100°C.
 - Contaminants are present. Check chemical compatibility charts (Section 4). Incompatible chemicals can interfere with the separation
 - Do not use autoclaved water for diluting protein samples or ladder
- **Poor molecular weight sizing:**
 - Ensure that the ladder peaks and upper and lower markers in ladder and sample wells were properly assigned by the software. Exclude peaks or manually assign markers if necessary
- **Poor quantitation:**
 - Ensure that the upper marker is correctly assigned by the software, and manually select if necessary

Section 6

Ordering Information

| Catalog # | Product Description |
|------------------|--|
| 700-7101 | Experion Pro260 Analysis Kit for 10 Chips , includes 10 Pro260 chips, 3 x 520 µl Pro260 gel, 45 µl Pro260 stain, 60 µl Pro260 ladder (10–260 kD), 400 µl Pro260 sample buffer, 3 spin filters |
| 700-7102 | Experion Pro260 Analysis Kit for 25 Chips , includes 25 Pro260 chips, 5 x 520 µl Pro260 gel, 2 x 45 µl Pro260 stain, 2 x 60 µl Pro260 ladder (10–260 kD), 2 x 400 µl Pro260 sample buffer, 5 spin filters |
| 700-7151 | Experion Pro260 Chips , 10 |
| 700-7152 | Experion Pro260 Reagents and Supplies , for 10 chips, includes 3 x 520 µl Pro260 gel, 45 µl Pro260 stain, 60 µl Pro260 ladder (10–260 kD), 400 µl Pro260 sample buffer, 3 spin filters |
| 700-7251 | Experion Cleaning Chips , 10 |
| 700-7254 | Experion Spin Filters , 10 |
| 700-7252 | Experion Electrode Cleaner , 250 ml |
| 163-2091 | ReadyPrep Proteomics Grade Water , 500 ml |
| 161-0710 | β-mercaptoethanol , 25 ml |

Section 7

Legal Notices

B-PER is a trademark of Pierce. Bug Buster is a trademark of Novagen. Coomassie is a trademark of BASF Alktingesellschaft. LabChip and the LabChip logo are trademarks of Caliper Life Sciences, Inc. Bio-Rad Laboratories, Inc. is licensed by Caliper Life Sciences, Inc. to sell products using the LabChip technology for research use only. Dye products are licensed under U.S. Patent Nos. US 5,863,753, 5,658,751, 5,436,134, and 5,582,947, and pending patent applications, and related foreign patents, for internal research and development use only in detecting, quantitating and sizing macromolecules, in combination with microfluidics, where internal research and development use expressly excludes the use of this product for providing medical, diagnostic, or any other testing, analysis or screening services, or providing clinical information or clinical analysis, in any event in return for compensation by an unrelated party. Triton is a trademark of Union Carbide. Tween is a trademark of ICI Americas, Inc.

Appendix Quick Guide

Equilibrating Kit Reagents

- Remove Pro260 stain, gel, sample buffer and ladder from 4°C storage and equilibrate to room temperature. Protect kit components from light at all times.
- Vortex tubes. Briefly centrifuge tubes to collect solutions.

Preparing the Gel-Stain and Sample Buffer

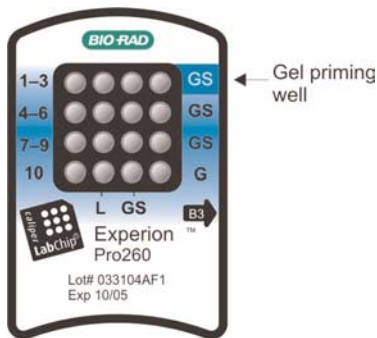
- Add 20 µl Pro260 stain (**blue cap**) to 520 µl Pro260 gel (**green cap**) in the gel tube provided. Vortex the gel-stain solution.
- Transfer the gel-stain solution (GS) and 520 µl gel (G) to separate spin filters and centrifuge at 10,000 × g for 5 min. **Note:** Filtered GS and filtered gel may be stored for up to 1 month at 4°C in the dark.
- For separations under reducing conditions, add 1 µl β-mercaptoethanol to 30 µl sample buffer (**yellow cap**) for each chip to be run. Prepare fresh solution daily and use it immediately. For separations under nonreducing conditions add 1 µl deionized water (0.2 µm filtered) to 30 µl sample buffer (**yellow cap**) for each chip to be run. Also prepare reducing sample buffer as instructed above as the ladder must be prepared under reducing conditions.

Preparing the Samples and Pro260 Ladder

- Add 2 μl sample buffer to 4 μl sample. Vortex.
- Add 2 μl sample buffer to 4 μl Pro260 ladder (**red cap**). Vortex.
- Heat the samples and ladder at 95-100°C for 3-5 min. Briefly centrifuge the samples.
- Add 84 μl deionized water (0.2 μm filtered) to samples and the ladder. Vortex.

Priming the Chip

- Remove a Pro260 chip from its packaging and place it on the chip platform in the Experion priming station
- Add 12 μ l gel-stain solution into the well labeled **GS** (gel priming well)
- Close the lid of the priming station, set the pressure to **B**, and set the time to **3**
- Press the **Start** button
- After priming is finished, remove the primed chip from the priming station and visually inspect the microchannels for trapped air bubbles or incomplete priming



Loading the Samples and Pro260 Ladder Into the Chip

- Remove the remaining gel-stain from the top right well labeled **GS** (gel priming well)
- Pipet 12 μ l filtered gel-stain solution into all four wells labeled **GS**
- Pipet 12 μ l filtered gel into the well labeled **G**
- Pipet 6 μ l diluted protein ladder into the well labeled **L**

- Pipet 6 μl diluted sample into each sample well. Do not leave any sample well empty. If necessary, pipet a sample or ladder replicate into any empty sample well
- Run the chip in the Experion electrophoresis station within 5 min of loading

Running the Pro260 Analysis

- Power the electrophoresis station on and launch the Experion software
- Place the loaded chip onto the electrophoresis station chip platform and close lid
- Select **New Run** and then **Experion Pro260**
- Click the **Start** button in the software to begin the run. Select the number of samples to run
- After the run is complete, remove and discard the used chip

Cleaning the Chip After a Run

- Place a cleaning chip filled with 800 μl water (0.2 μm filtered) into the electrophoresis station
- Close the lid for 60 sec to clean the electrodes, and then open it for 60 seconds to allow the electrodes to completely dry
- Remove the cleaning chip



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