Experion™ Pro260 Starter Kit

Instruction Manual

Catalog Number

700-7110

BIO-RAD

About This Manual

This manual serves as an instruction manual for the Experion™ Pro260 starter kit. General instructions for using the Experion system and the Pro260 analysis kit, and for analyzing protein separation data, can be found in the following:

- The Experion Pro260 analysis kit instruction manual (Bio-Rad bulletin 10000975)
- The Experion system instruction manual (Bio-Rad bulletin 10001312)

In addition, a number of tech notes, product information sheets, and fliers provide more details about the features and applications of the Experion system. For a complete, up-to-date list of all Experion literature, visit www.bio-rad.com/experion/

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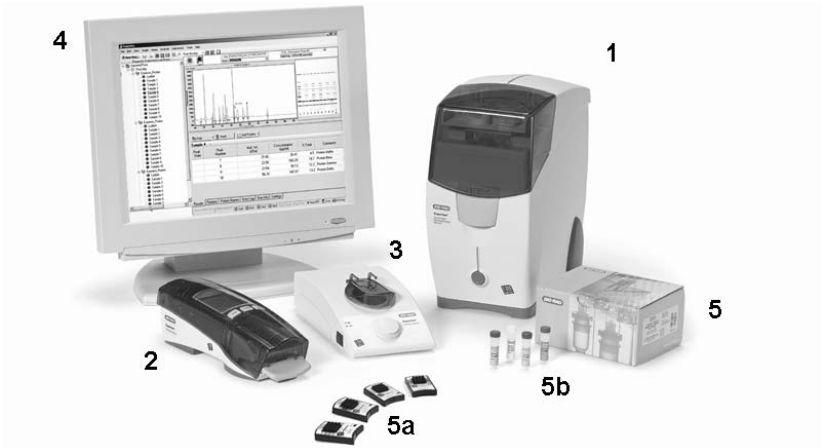
Introduction — Product Description and Components

Experion™ System and Pro260 Starter Kit

The Experion automated electrophoresis system employs LabChip microfluidic technology to automate protein and nucleic acid electrophoresis and analysis. This system integrates separation, detection, and data analysis within a single platform and uses smaller sample and reagent quantities than standard analysis methods. The Experion system accomplishes analysis in a single 30 minute, automated step.

The Experion electrophoresis system includes the following components:

- Automated electrophoresis station
- Microfluidic chips
- Priming station
- Vortex station (for RNA and DNA analyses only)
- Reagents (analysis kits and individual components)
- Experion software



The Experion system includes the following components: 1) automated electrophoresis station, 2) priming station, 3) vortex station used for nucleic acid analysis only, 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), high-sensitivity RNA (HighSens kit), and DNA (DNA 1K and 12K kits) analyses.

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, which connect with the base of the plastic wells. The channels are primed with a gel matrix, the samples are applied to the appropriate wells, and the electrophoresis station directs the samples through these microchannels by controlling the voltages and currents that are applied. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software accomplish separation, staining, destaining, detection, and basic data analysis without any user intervention.

The Experion Pro260 starter kit was designed to introduce new users to protein analysis with the Experion system as well as for performing troubleshooting, performance maintenance, or performance qualification of the Experion system. The starter kit is derived from the Experion Pro260 analysis kit, which is used with the Experion system to perform separation and analysis of proteins between 10–260 kD under denaturing conditions. The starter kit includes the Experion Pro260 ladder, which contains nine highly purified recombinant proteins with molecular masses from 10 to 260 kD and has been optimized for automated electrophoresis on the Experion system. The kit also features the Pro260 sample buffer, which contains a lower marker and an upper marker used for the proper alignment of samples to the Pro260 ladder. The starter kit contains all of the components of the Experion Pro260 analysis kit in addition to other supplies that allow a new user to start using the system immediately.

For more information on protein analysis using the Experion system, refer to Appendix A of this manual.

Kit Components and Storage Conditions

Experion Pro260 Starter Kit Components and Storage Conditions

Component	Storage Conditions
Experion Pro260 chips, 3	Ambient
Reagents for analysis	4°C
Pro260 gel, 2 vials, 520 µl each	
Pro260 ladder, 1 vial, 60 µl	
Pro260 sample buffer, 400 µl	
Pro260 stain, 45 µl	
Spin filters, 2	
Bovine γ-globulin (BGG) standard (2.0 mg/ml), 2 ml	4°C
Dithiothreitol (DTT)	4°C
DEPC-treated[1] water, 50 ml	Ambient
Experion electrode cleaner, 25 ml	Ambient
Experion cleaning chip, 1	Ambient
Foam swabs, 25	Ambient
Narrow-bore 20 µl pipet tips, 96 (1 rack)	Ambient
0.65 ml RNase-free microcentrifuge tubes, 50	Ambient
Experion Training Video: Chip Loading (DVD)	Ambient
Instructions	Ambient

[1] DEPC treatment of water is not required for the Pro260 assay. Use ultrapure (at minimum 0.2 µm-filtered) water. Do not use autoclaved water.

Additional Materials and Equipment Required

- Experion automated electrophoresis station
- Experion priming station
- Microcentrifuge (1,000–10,000 x g)
- Aluminum foil
- Heating block or water bath set at 95–100°C
- Benchtop vortexer
- Extra spin filter for additional filtration steps (optional, catalog #700-7254)
- Ice

The Experion Pro260 Starter Kit Tests

The Experion Pro260 starter kit includes all the reagents, consumables, and instructions required to perform three tests, each of which demonstrates a key protein analysis function of the Experion system. Each test uses various dilutions and preparations of a standard protein, bovine γ-globulin (BGG), and each test follows the standard Experion Pro260 assay workflow, which is summarized below.

Experion Pro260 Starter Kit Tests

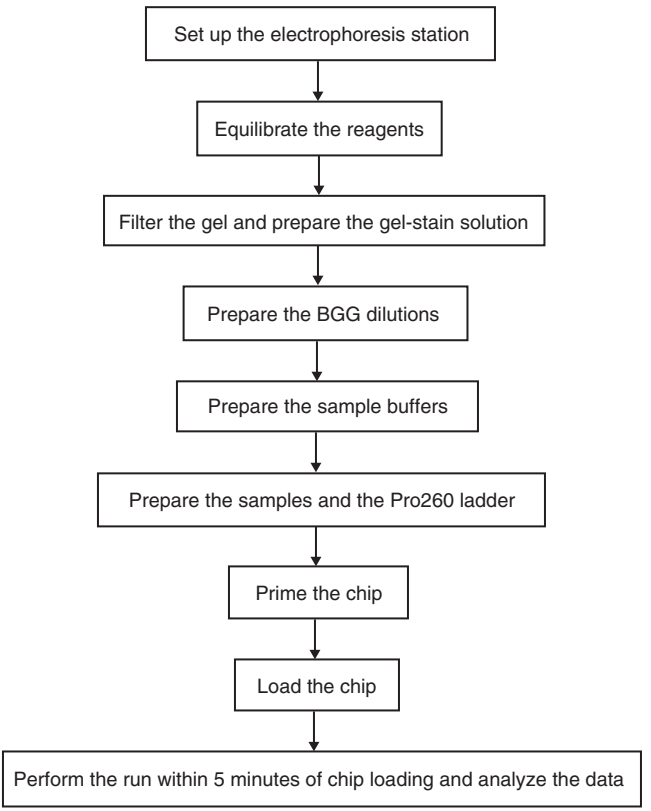
Test	Name	Purpose	Description
1	Sizing and relative quantitation	Used for familiarization with the Experion system and for troubleshooting. Demonstrates the basic functions of a Pro260 assay and how to perform relative quantitation, the easiest, fastest method of protein concentration determination. Also demonstrates protein sizing and how to determine the accuracy and reproducibility of a Pro260 analysis	BGG samples are analyzed using standard nonreducing conditions and are quantitated using relative quantitation against the upper marker
2	Percentage determination	Used for demonstration of the percentage determination function of Experion software, which can be used for rapid evaluation of sample purity	BGG samples are analyzed under reducing conditions to allow separation of the BGG subunits. The heavy- and light-chain fragments are used to demonstrate the percentage determination calculations
3	Absolute quantitation using a calibration curve	Used for demonstration of the absolute quantitation method, which involves creation of a calibration curve. Demonstrates how to generate and evaluate the linearity of a calibration curve and how to determine the accuracy and reproducibility of absolute quantitation. The results demonstrate how use of a calibration curve can significantly improve quantitation accuracy	Five BGG samples at various concentrations are used to generate the calibration curve used for quantitation of another BGG sample

Before running any of the tests:

- Watch the Experion Training Video: Chip Loading (supplied with this kit and available at www.bio-rad.com/experion/)
- Clean the electrodes as detailed in Appendix B

Note: If this is the first time the Experion electrophoresis station is being used, refer to Appendix C for instructions on how to prepare the system for use.

Experion Pro260 Starter Kit Test Workflow



Starter Kit Test 1 — Sizing and Relative Quantitation

1.1 Overview of Test 1

Microfluidic chips use much smaller volumes of samples and reagents than traditional slab gels. As a result, microfluidic separations are much more sensitive to variability in pipetting technique when compared to traditional electrophoresis methods. Proper pipetting skills and tools are critical to a successful Experion™ analysis.

In this test, you prepare a dilution series of the bovine γ-globulin (BGG) protein, run an Experion Pro260 analysis of the series, check the resulting electropherograms for proper performance, examine protein sizing and relative quantitation results, and export the data to determine the accuracy and reproducibility of the analysis.

Since the use of proper pipet tips can also impact assay performance, use the narrow-bore pipet tips included with the Experion Pro260 starter kit (or similar narrow-bore tips) for loading samples and reagents into the microfluidic chip. Narrow-bore tips are required for successful chip loading (other tips can introduce bubbles during chip loading, leading to problems in performing the assay or in the quality of results).

1.2 Assay Procedure

1.2.1 Set Up the Electrophoresis Station

Note: If this is the first time the Experion electrophoresis station is being used, refer to Appendix C for instructions on how to prepare the system for use.

1. Power on the computer.
2. Power on the Experion electrophoresis station. Push the green button in the center of the front panel. The steady green LED above the button indicates that the unit is on.
3. Launch Experion software. Check the screen to confirm that the instrument and computer are communicating properly.

   When communication has been established:

   - A green dot with the last four digits of the instrument serial number appears in the lower right corner of the main software screen
   - The electrophoresis station icon appears in the upper left corner

   When there is no connection, these indicators are absent and a “disconnected” message appears next to the **Start Run** button  in the upper left corner of the screen. In addition, a grayed-out instrument icon appears in the upper left corner of the software screen.

1.2.2 Equilibrate the Kit Reagents

1. Set a heating block or water bath to 95–100°C. You will use this heating block to denature the samples and the Pro260 ladder later in the protocol.
2. Remove the kit components from storage.

- Keep the BGG standard on ice
- Equilibrate the following to room temperature for 15–20 min:
  - Dithiothreitiol (DTT)
  - Pro260 sample buffer (yellow cap)
  - Pro260 ladder (red cap)
  - 2 Tubes of Pro260 gel[2] (green cap)
  - Pro260 stain (blue cap)[2]

Notes: Keep the Pro260 stain (blue cap) and sample buffer (yellow cap) covered (for example, with foil) at all times to avoid exposure to light.

Inspect the reagents; if the Pro260 gel (green cap) has been frozen, do not use it.

Do not store reagents at room temperature for more than 2 hr, as this will affect their shelf life.

3. Vortex the contents of each tube and briefly centrifuge to collect the solutions at the bottoms of the tubes. Make sure that the Pro260 stain solution (blue cap) is completely thawed before proceeding.

1.2.3 Filter the Gel and Prepare the Gel-Stain Solution

Notes: The Experion Pro260 starter kit includes 2 spin filters, which are sufficient for preparation of one filtered gel (G) and one gel-stain solution (GS). You will use the G and GS in Tests 2 and 3. The prepared G and GS can be used for up to 4 weeks when stored in the dark at 4°C between each use. The solutions can be refiltered once after that to extend their use for another 4 weeks.

Additional spin filter tubes and reagents may also be purchased separately (see Appendix D for ordering information).

Prepare GS as described below. Do not prepare smaller aliquots, as doing so may adversely affect the gel-to-stain ratio, which is critical to successful staining.

1. Prepare the GS by adding 20 µl Pro260 stain (blue cap) to a tube of 520 µl Pro260 gel (green cap). (Each tube of Pro260 gel contains 520 µl gel.) Vortex the GS for 10 sec at the highest setting and then spin it down in a microcentrifuge for a few seconds.

Note: Cap the Pro260 stain tightly (it contains DMSO, which is highly hygroscopic), and store the tube in the dark (for example, cover it with foil) at 4°C.

2. Transfer the GS to a spin filter. Label the tube with “GS” and the date.
3. Pipet the contents (520 µl) of the other tube of Pro260 gel (green cap) into another spin-filter tube. Label the tube with “G” and the date.
4. Centrifuge both spin filters for 5 min at 10,000 × g.
5. Inspect the tubes to ensure that all of the gel has passed through the filters and then discard the filters.
6. Keep both prepared G and GS at room temperature and covered until ready for use.

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[2] If the filtered gel (G) and gel-stain solution (GS) were prepared previously, remove these from storage and equilibrate them to room temperature. Do not remove the Pro260 stain.

1.2.4 Prepare the BGG Dilutions

Prepare a serial dilution of the bovine γ-globulin (BGG, 2.0 mg/ml) supplied with the kit. Label three 0.65 ml microcentrifuge tubes 1–3, and add 200 µl DEPC-treated water into each tube. Then, add the following:

Tube 1 (1,000 ng/µl):	200 µl BGG stock solution
Tube 2 (500 ng/µl):	200 µl BGG dilution from tube 1
Tube 3 (250 ng/µl):	200 µl BGG dilution from tube 2

Notes: You may also use samples 1 and 2 in Test 2[3]. If running samples over multiple days, store these samples at 4°C between uses.

1.2.5 Prepare the Sample Buffers

Protein samples can be separated under either reducing or nonreducing conditions, but the Pro260 ladder must always be run under reducing conditions. Reduced and nonreduced samples may be run on the same chip. Prepare fresh sample buffer each day that an assay is run.

The starter kit includes dithiothreitol (DTT) in place of the β-mercaptoethanol specified in the Experion Pro260 analysis kit instruction manual. For the purpose of Experion Pro260 analysis, these reducing agents are interchangeable.

1. Reconstitute the DTT by adding 1 ml DEPC-treated water. This solution remains stable for weeks after preparation if stored at –20°C.
2. Transfer 30 µl sample buffer (yellow cap) to a 0.65 ml microcentrifuge tube. Add 1 µl of the reconstituted DTT solution, and label this tube “R” for “reducing”.
3. Transfer another 30 µl sample buffer (yellow cap) to another 0.65 ml microcentrifuge tube. Add 1 µl DEPC-treated water, and label this tube “NR” for “nonreducing”.

Note: When you are finished preparing the sample buffers, return the stock sample buffer (yellow cap) to storage at 4°C and the reconstituted DTT to storage at –20°C. Protect all tubes containing sample buffer from direct sunlight.

[3] Do not use these samples for Test 3. For Test 3, prepare the entire dilution series as directed and at the same time for best results.

1.2.6 Prepare the Samples and the Pro260 Ladder

1. Prepare the Pro260 ladder. Mix 4 µl Pro260 ladder (red cap) and 2 µl sample buffer "R" (reducing sample buffer) in a 0.65 ml microcentrifuge tube. Vortex the tube briefly and spin down for a few seconds. Label the tube "L".
2. Prepare the samples (S1–S3). Label three 0.65 ml microcentrifuge tubes "S1", "S2", and "S3". For each sample, combine 4 µl of the BGG samples in tubes 1–3 (from Section 1.2.4) with 2 µl sample buffer "NR" (nonreducing).
3. Vortex all tubes briefly and spin down in a microcentrifuge for a few sec.
4. Place the sample tubes S1–S3 and the Pro260 ladder (L) in a 95–100°C heating block for 3–5 min.
5. Spin the tubes for 15 sec, add 84 µl DEPC-treated water to each tube, and vortex briefly to mix.

1.2.7 Prime the Chip

Before priming the chip, be sure that all samples, the Pro260 ladder, and other reagents are prepared and ready to be loaded. If a chip is not used within 5 min of priming and loading, reagents may evaporate, leading to poor results. Use the narrow-bore pipet tips that are supplied with the kit (or equivalent tips) when loading samples and reagents into the chip.

1. Pipet 12 µl GS into the top right well labeled **GS** (gel priming well) (Figure 1.1). Insert the pipet tip vertically and to the bottom of the well when dispensing. Dispense slowly, and do not expel air at the end of the pipetting step.

Note: 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. Dislodge any bubbles at the bottom of the well with a clean pipet tip, or remove the GS and load it again. For help with chip loading, refer to the Experion Training Video: Chip Loading.

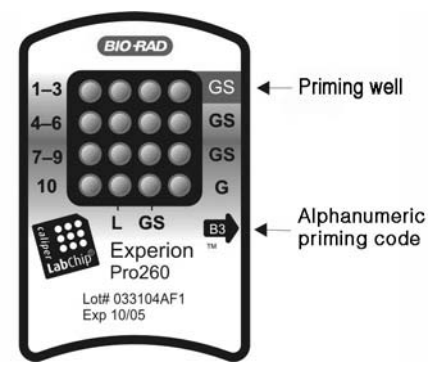


Fig. 1.1. Experion Pro260 chip. The locations of the gel priming well (GS) and alphanumeric priming code are indicated.

2. Set the pressure setting to **B** and the time setting to **3**, as specified by the alphanumeric code on the chip (Figure 1.1).
3. Open the Experion priming station by pressing down on the lever on the front.
4. Place the chip 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.
5. Carefully close the priming station by pressing down on the lid. The lid should snap closed.
6. Press the **Start** button. A “Priming” message appears on the screen of the priming station, the priming station pressurizes, and the timer begins to count down. Priming requires approximately 60 sec to complete. Do not open the priming station during the countdown.
7. An audible signal and “Ready” message indicate that priming is complete. Open the priming station by pressing down on the release lever.

Note: If the lid sticks, press down on it while pressing down on the release lever.

8. Remove the chip from the priming station, turn it over, and inspect the microchannels for bubbles or evidence of incomplete priming. If the chip is primed properly, the microchannels are difficult to see (it may be helpful to compare a primed chip to a new, unused chip). If you detect a problem, such as a bubble or incomplete priming, prime a new chip.
9. Place the chip on a clean surface for loading.

1.2.8 Load the Chip

1. Using a pipet, remove and discard any remaining GS from the gel priming well (Figure 1.1).
2. Pipet 12 µl GS into all four wells labeled **GS** (including the gel priming well) (Figure 1.2).
3. Pipet 12 µl filtered gel (G) into the well labeled **G** (Figure 1.2).

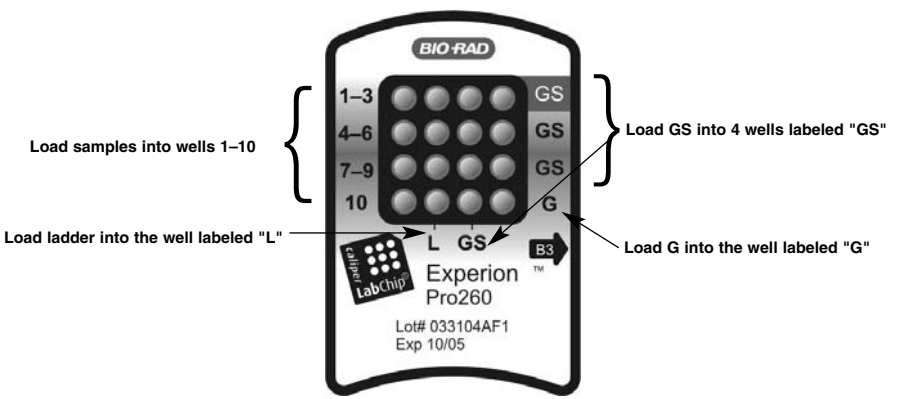


Fig. 1.2. Experion Pro260 chip. GS, gel-stain solution; G, gel; 1–10, samples; L, Pro260 ladder.

4. Pipet 6 µl of each diluted sample into sample wells 1–10. Use the layout shown in Figure 1.3.

S3	S2	S1	GS
S3	S2	S1	GS
S3	S2	S1	GS
S3	L	GS	G

Fig. 1.3. Chip layout for Test 1. S1–S3, sample numbers; GS, gel-stain solution; G, Pro260 gel; L, Pro260 ladder.

5. Inspect all wells to make sure that there is no excessive bubble formation from pipetting. Hold the chip above a light-colored background and look down through the wells (Figure 1.4). Dislodge any bubbles at the bottom of the well with a clean pipet tip or by removing and reloading the solution.
6. Pipet 6 µl diluted Pro260 ladder into the ladder well labeled **L** (Figure 1.3). Use the Pro260 ladder within 8 hr of preparation. Every chip **must** have the Pro260 ladder loaded into the ladder well labeled **L**.

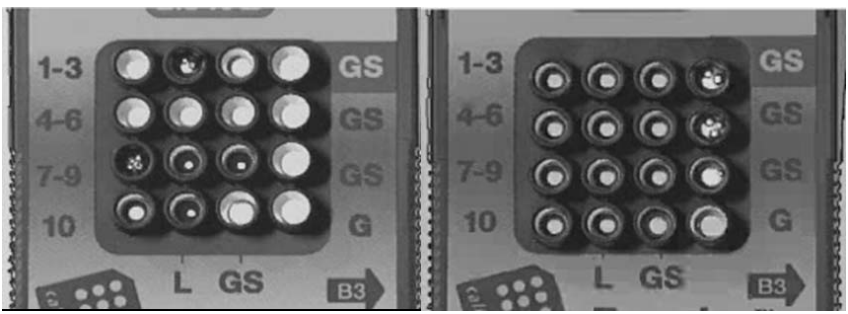


Fig. 1.4. Bubble formation during loading of Experion Pro260 chips. Left, example of bubbles trapped at the bottom of wells. The GS and G wells and sample wells 1, 3, and 4–6 contain no solution. Well 10 is filled properly and has no bubbles; large bubbles have formed at the bottom of wells 8 and 9 and in the ladder well (L). Note the difference in the diameter of the light-colored circles in wells 10 and L. Right, example of bubble formation at the surface of wells. Small bubbles have formed at the surface of the three GS wells on the right side of the chip, and the rest of the wells have no bubbles. Surface bubbles should not cause problems during a run, but bubbles at the bottoms of wells must be removed.

7. Place the loaded chip into the Experion electrophoresis station and start the run. It is important to run a loaded chip immediately (within 5 min). Otherwise, excess evaporation may occur, leading to poor results or to a chip performance error.

1.2.9 Run the Pro260 Analysis

1. Open the lid of the electrophoresis station by pulling the release latch. Place the primed and loaded chip on the chip platform and close the lid.

2. In the Experion software toolbar, click the **New Run** button  . The **New Run** screen opens (Figure 1.5).

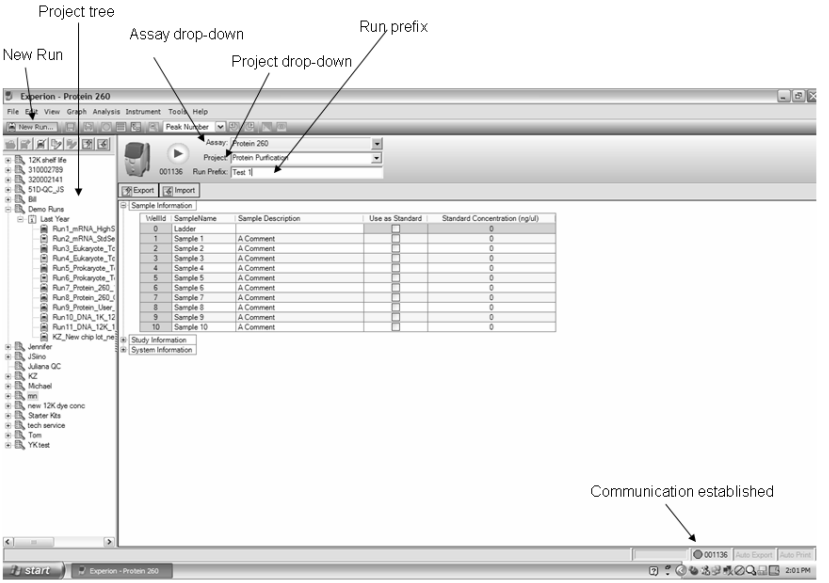


Fig. 1.5. Details of the New Run screen. The green dot in the lower right corner indicates that communication between the electrophoresis station and Experion software has been established.

3. From the **Assay** drop-down list, select **Protein > Pro260**.
4. Select a project folder for the run from the **Project** drop-down list.

   -Or-

   Create a new project folder by typing a name in the **Project** field or by selecting **File > Project > New**.

   The project folder appears in the project tree after the run has started.
5. Enter a name for the run in the **Run Prefix** field.

6. Click the **Start Run** button  at the top of the window to start the run. The **New Run** dialog opens (Figure 1.6).

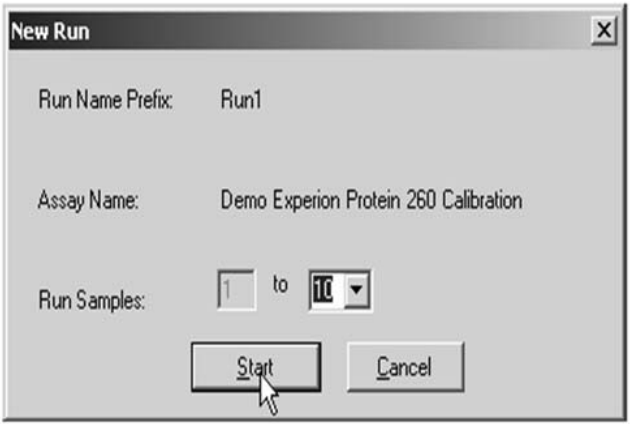


Fig. 1.6. New Run dialog. The Experion system stops analysis when it reaches the number of samples entered.

7. Select the number of samples to be analyzed (10 for all three tests).
8. Click **Start** to begin the chip run. The green LED in the center of the front panel on the electrophoresis station blinks once the run has started.

Warning: Do **not** open the lid of the Experion electrophoresis station until the run is complete. The lid does not lock. Opening the lid prematurely aborts the run.

The system performs a number of checks at the beginning of a run: it confirms that a chip has been inserted, that all wells contain liquid, that electrical connections are made, etc. A calibration counter marks the progress of these calibrations in the upper right corner of the screen.

Note: An "IV Check Error" message indicates the system cannot make electrical contact in one or more of the wells. This often means that either there is a bubble at the bottom of a well or that a well is empty. If this message appears, abort the run, and check the chip for bubbles or empty wells. Refill the affected well(s), and start the run again.

9. Enter the sample names in the **Sample Information** window. For example, for wells 1, 4, 7, and 10, enter "S3". In addition, add sample details, such as sample concentration. Alternatively, enter this information after the run is complete using the **Run Info** tab.
10. During separation, the sample name is highlighted in gray in the project tree, and the electropherogram trace and virtual gel bands appear in real time. By default, the electropherogram of the sample being separated appears in the electropherogram view; in the gel view, the lane corresponding to that sample is outlined in pink and has a dark background. To display the electropherogram from another sample that has already been separated, click on either the sample name in the project tree or on a lane in the virtual gel.
11. When the analysis is complete (after approximately 30 min), the instrument beeps and a window opens indicating the end of the run. Select **OK** to the prompt and remove the Pro260 chip from the chip platform.

12. Clean the electrodes using the DEPC-treated water provided in the kit (see Appendix B, routine cleaning). DEPC-treated water is not required for this cleaning application; however, always use ultrapure (at minimum 0.2 µm-filtered) water.
13. Analyze the run and the data.

1.3 Data Analysis

Evaluate the performance of Test 1 and the analysis of the data by the Experion software.

1. Evaluate the virtual gel to ensure that all lanes (samples) are visible. Check that the upper and lower markers are present in each sample (indicated by pink triangles) and that they are aligned across all lanes (Figure 1.7).
2. Modify the gel view by changing the fluorescence intensity scale. By default, Experion software displays the virtual gel and electropherograms as “scaled to local”, which means the gel lane or trace of each sample is scaled to the highest peak intensity detected in that sample. This is the best method for viewing an electropherogram. To adjust the fluorescence intensity scale so that the virtual gel resembles an SDS-PAGE separation, select **Graph > Scale to Global**. The virtual gel appears as it does in Figure 1.7, right panel. All lanes are scaled to the highest peak found across the entire chip, which enables better definition of the concentration differences among the samples in the virtual gel.

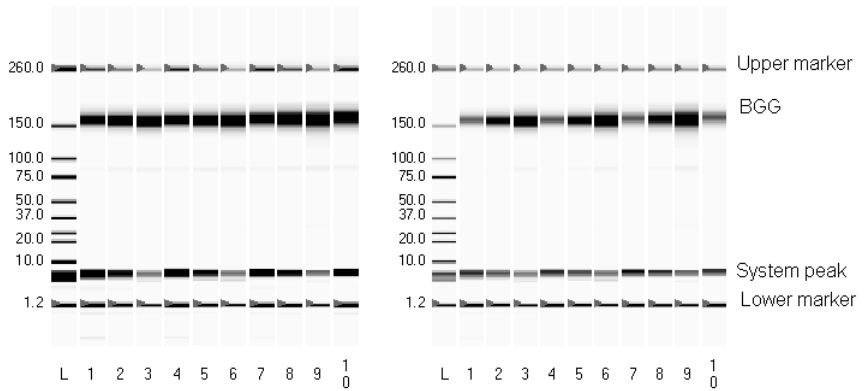


Fig. 1.7. Virtual gel generated by Test 1. Left, virtual “scaled to local”. Right, virtual gel “scaled to global”. L, Pro260 ladder; lanes 1, 4, 7, and 10, 250 ng/µl BGG; lanes 2, 5, and 8, 500 ng/µl BGG; lanes 3, 6, and 9, 1,000 ng/µl BGG. Using the “scale to global” option allows clearer distinction of the concentration differences among samples.

3. Evaluate the separation of the Pro260 ladder (Figure 1.8). This is a critical step in data analysis because much of the automated data analysis performed by Experion software is based on the successful separation of the Pro260 ladder. To display the ladder electropherogram, click the ladder well in the project tree or click on the lane labeled **L** in the virtual gel. The electropherogram should resemble the one shown below and should have the following features:
    - Two marker peaks and one set of system peaks
    - 8 Pro260 ladder peaks located between the system peaks and upper marker

- Flat baseline
- Marker peaks at at least 20 fluorescence units above the baseline

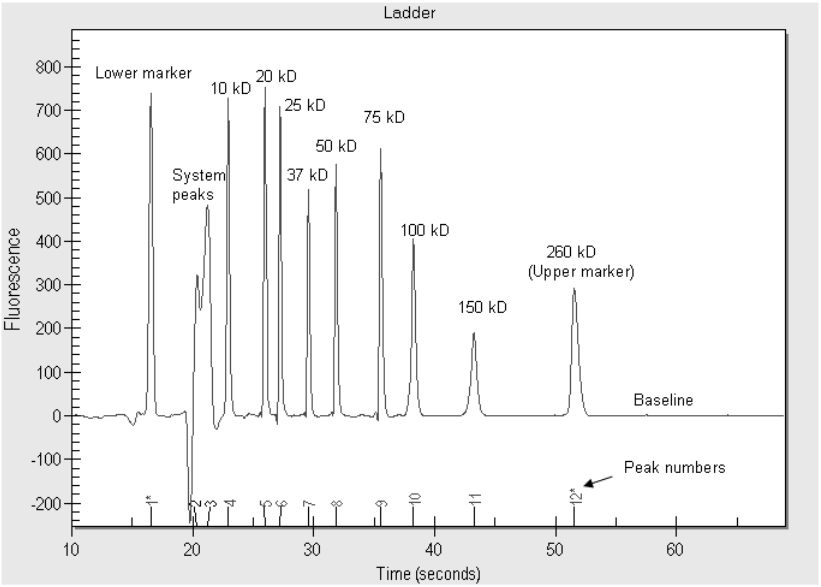


Fig. 1.8. Electropherogram showing the separation of the Pro260 ladder. Note the flat baseline and well-resolved peaks. All identified peaks are numbered, and the lower and upper markers are indicated by green asterices. The 260 kD protein in the Pro260 ladder is labeled as the upper marker by Experion software.

4. Examine the separation of the 1,000 ng/µl BGG sample (S1). Click on the sample name in the project tree or on lanes 3, 6, or 9 in the virtual gel. The electropherogram appears and should have the following features (Figure 1.9):
   - Two well-resolved marker peaks and one set of system peaks
   - Sample peaks located between the system peaks and upper marker
   - Flat baseline
   - Marker peaks at least 20 fluorescence units above the baseline

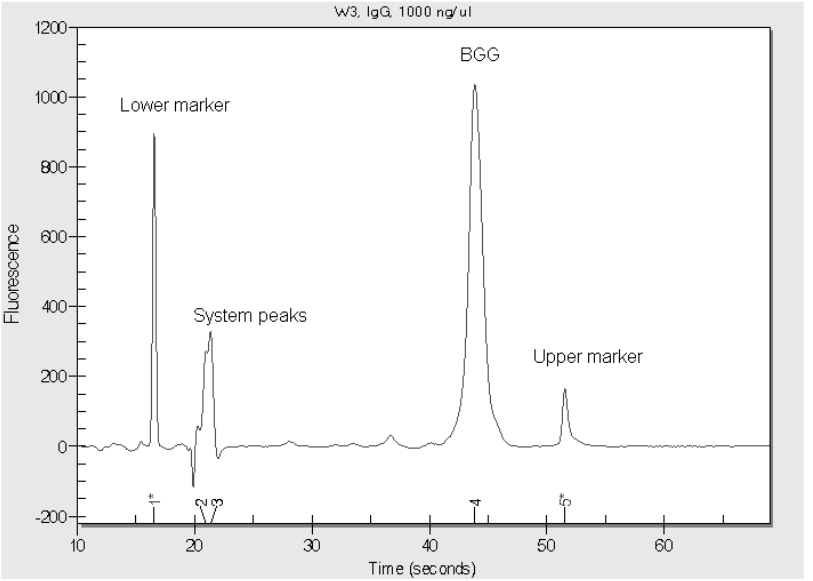


Fig. 1.9. Electropherogram showing the separation of the 1,000 ng/µl sample under nonreducing conditions.

5. Peaks identified by Experion software are automatically labeled with numbers for easy identification, and the upper and lower markers used for normalization are indicated in green with an asterisk (*). To change the type of label displayed, select **Graph > Peak Info** or use the drop-down menu and select among the following options:

   Peak number — uses sequential numbers for peak identification (default selection)

   Peak time — uses peak migration time (min:sec) for the peak labels

   Peak height — uses peak height in units of fluorescence intensity for peak labels

   Peak corrected area — uses calculated (corrected) peak area for peak labels

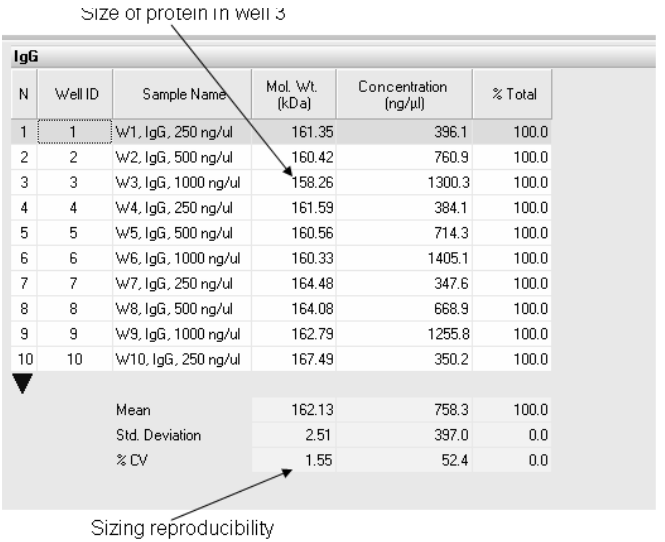
   Molecular weight — uses calculated molecular weight (kD) for peak labels

   Peak concentration — uses calculated concentration (ng/µl) for peak labels

6. Evaluate the data analysis performed by Experion software. Click on the **Results** tab and click on any sample in the virtual gel to view the data for that sample.
   - To evaluate sizing, examine the values provided in the **Mol. Wt. (kD)** column for each sample. BGG is peak 4 in the electropherogram in Figure 1.9 (well 3), and the peak size is ~158.26 kD.
   - To evaluate relative quantitation of the BGG peak, examine the values provided in the **Concentration** (**ng/µl**) column for each sample.

7. Evaluate the reproducibility of the sizing calculation using the coefficient of variation (%CV), which should be ≤10%. Click on the **Protein Search Criteria** tab and click on the **Add Protein Name** icon . Replace **Change Protein Name 1** with ***BGG*** in the **Protein Name** column, enter ***160*** in the **Mol Wt.** column, and enter ***16*** in the **± kDa** column.

8. Click the **Protein Search Results** tab and scroll down to find the %CV calculation. In the example given in Figure 1.10, the %CV is 1.55%.



N	Well ID	Sample Name	Mol. Wt. (kDa)	Concentration (ng/µl)	% Total
1	1	W1, IgG, 250 ng/ul	161.35	396.1	100.0
2	2	W2, IgG, 500 ng/ul	160.42	760.9	100.0
3	3	W3, IgG, 1000 ng/ul	158.26	1300.3	100.0
4	4	W4, IgG, 250 ng/ul	161.59	384.1	100.0
5	5	W5, IgG, 500 ng/ul	160.56	714.3	100.0
6	6	W6, IgG, 1000 ng/ul	160.33	1405.1	100.0
7	7	W7, IgG, 250 ng/ul	164.48	347.6	100.0
8	8	W8, IgG, 500 ng/ul	164.08	668.9	100.0
9	9	W9, IgG, 1000 ng/ul	162.79	1255.8	100.0
10	10	W10, IgG, 250 ng/ul	167.49	350.2	100.0
		Mean	162.13	758.3	100.0
		Std. Deviation	2.51	397.0	0.0
		% CV	1.55	52.4	0.0

Fig. 1.10. Results table from Test 1 showing reproducibility of protein sizing. In this example, the %CV for sizing is 1.55%. Note that the apparently large %CV for concentration is misleading (different sample concentrations were analyzed).

9. To perform a qualitative evaluation of quantitation reproducibility, overlay the electropherograms of replicate samples. For example, in the virtual gel, select lane 3 and hold the Ctrl key while selecting lanes 6 and 9. The electropherograms from these wells (1,000 ng/µl samples) overlap (Figure 1.11). Tighter overlaps signify better reproducibility. Repeat this step for the other two concentrations examined.

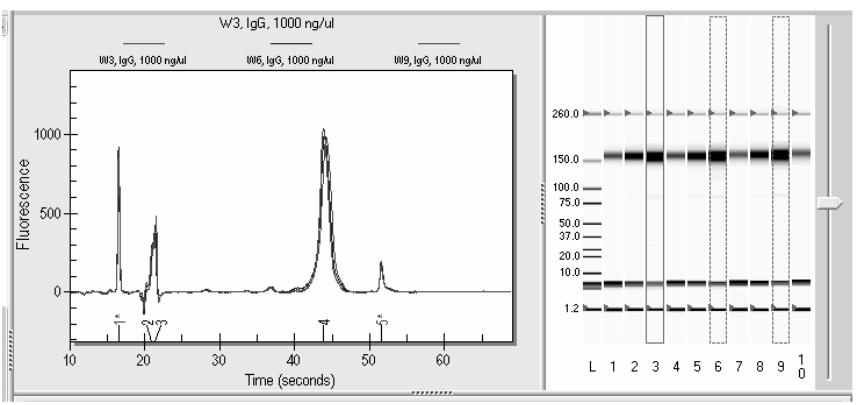


Fig. 1.11. Electropherogram overlay of the 1,000 ng/µl samples. The virtual gel (right) shows that the samples in wells 3, 6, 9 are being compared.

10. The relative concentration of the BGG, as determined against the known concentration of the upper marker, is shown in the **Concentration** (**ng/µl**) column. To perform an evaluation of quantitation reproducibility, calculate the %CV, which is defined as the standard deviation/mean x 100. To do this, export the data to a Microsoft Excel or other spreadsheet application either by entering the values manually in Excel or by using the data export feature of Experion software.
11. To export the Protein Table data:

    Click the **Protein Search Results** tab. Right-click on the table, select **Copy Table(s)** > **Selected Table to Clipboard**, and paste into an Excel spreadsheet. Delete the three lines of calculation at the bottom.

    -Or-

    Select **File > Export Data…** The **Export** window opens. Deselect **Result Table**, and select **Protein Table**. Click **Export**.

    In the **Save As** window, name the file and designate a folder for it. Click **Save**.

    Open the file in Excel software. Delete the three lines of calculation at the bottom.
12. In the Excel spreadsheet, sort the data by protein concentration. Insert a column between columns D and E, and label it ***Exp Conc*** (Expected Concentration). Enter the expected concentration for each well (enter ***250*** for wells 1, 4, 7, and 10; ***500*** for wells 2, 5, and 8; ***1,000*** for wells 3, 6, and 9). Do not enter units, as these will interfere with the sort function. Place the cursor on column E (Exp Conc) and select **Data > Sort**.
13. Insert 3 columns after column G, and label them ***Mean Conc***, ***Stdev***, and ***%CV***. Use Excel software to determine the mean, standard deviation, and the %CV (standard deviation/mean x 100) for each set of replicates (each concentration examined). The %CV for this test should be ≤20%.

Expected Performance of BGG in Test 1

Measurement	Typical Results
Sizing	
Reproducibility	%CV ≤ 10%
Accuracy	160 ± 16 kD
Relative quantitation	
Reproducibility	%CV ≤ 20%

Notes: The Experion system uses a single-point calibration to the upper marker protein for relative quantitation; therefore, relative quantitation accuracy is often highest for proteins that stain with similar efficiency to the upper marker protein. Though the accuracy of this method for some proteins can be high (within <0.5% of expected)[4], accuracy for other proteins, such as the BGG standard protein, may not be as good. Such differences in quantitation accuracy for different proteins also occur with other commonly used protein quantitation methods, such as the Bradford and Lowry assays[5–8], and are often due to differences in protein staining efficiency; unique protein characteristics can affect the levels of staining and colorimetric signals produced.

Because proteins exhibit differences in staining behavior, alternative protocols exist for their quantitation: relative quantitation against a user-defined internal standard and absolute quantitation. Perform Test 3 to see how absolute quantitation improves quantitation accuracy for the BGG standard. For more information on the quantitation methods used by Experion software, refer to Appendix A.

If your results do not appear as they do in these examples, refer to the troubleshooting section of the Experion Pro260 analysis kit and Experion system instruction manuals. Typically, problems with reproducibility are due to problems with pipetting or chip loading. For tips on optimizing your results, refer to the Experion Training Video: Chip Loading and the Essential Practices and Troubleshooting sections of the Experion system instruction manual.

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4 Nguyen M and Strong W, Performance comparison of the Experion automated electrophoresis system and a competing automated system for protein analysis, Bio-Rad bulletin 5302 (2005)

5 Bio-Rad bulletin 1069, Colorimetric protein assays

6 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem 72, 248–254 (1976)

7 Lowry OH et al., Protein measurement with the Folin reagent, J Biol Chem 193, 265–275 (1951)

8 Wu F and Strong W, Comparison of protein quantitation methods using the Experion automated electrophoresis system, BioRadiations 123, 28–31 (2007)

Starter Kit Test 2 — Percentage Determination

2.1 Overview of Test 2

In this test, you examine the separation of the BGG standard under reducing conditions and different total protein concentrations. Under reducing conditions, BGG separates into light- and heavy-chain fragments. These fragments are used to highlight the percentage determination function of Experion™ software, which calculates each fragment as a percent of the total protein species in a sample. This quantitation method is fast, reliable, accurate, and easy to perform; it is particularly useful for routine quality control experiments to evaluate the purity of known proteins.

In this test, note that the relative abundance of light- and heavy chain fragments does not change, regardless of the total protein concentration analyzed.

2.2 Assay Procedure

1. Set up the electrophoresis station, equilibrate the reagents to room temperature, and prepare the gel (G) and gel-stain solution (GS) as outlined in Sections 1.2.1–1.2.3. If the G and GS have already been prepared, equilibrate them as detailed in Section 1.2.2.
2. This protocol uses 1,000 and 500 ng/µl dilutions of BGG. Label 2 microcentrifuge tubes (1–2) and add 200 µl DEPC-treated water into each tube. Then add the following:

   Tube 1 (1,000 ng/µl): 200 µl BGG stock solution

   Tube 2 (500 ng/µl): 200 µl BGG dilution from tube 1

Note: If you have recently run Test 1 or 3 (within a month and using proper storage at 4°C), you can use the 1,000 and 500 ng/µl dilutions prepared for those tests in this test.

3. Prepare the reducing sample buffer (“R”) as described in Section 1.2.5. For this test, do not prepare nonreducing buffer (“NR”). Prepare fresh sample buffer each day that an assay is run.
4. Prepare the samples S1 and S2 and the Pro260 ladder as described in Section 1.2.6, except use reducing buffer (“R”) for preparing both the samples and the Pro260 ladder.
5. Prime and load the chip as described in Sections 1.2.7 and 1.2.8 and using the chip layout shown in Figure 2.1.

S2	S1	S2	GS
S1	S2	S1	GS
S2	S1	S2	GS
S1	L	GS	G

Fig. 2.1. Chip layout for Test 2. S1–S2, sample numbers; GS, gel-stain solution; G, Pro260 gel; L, Pro260 ladder.

6. Run the Pro260 analysis as described in Section 1.2.9. Enter the sample names and information appropriate to the chip layout for Test 2 (Figure 2.1) .

2.3 Data Analysis

Evaluate the run and the analysis of the data by the Experion software.

1. Evaluate the virtual gel to ensure that all lanes (samples) are visible. Check that the upper and lower markers are present in each sample (indicated by pink triangles) and that they are aligned across all lanes. Select **Graph > Scale to Global** to adjust the virtual gel so that it resembles an SDS-PAGE separation (Figure 2.2).

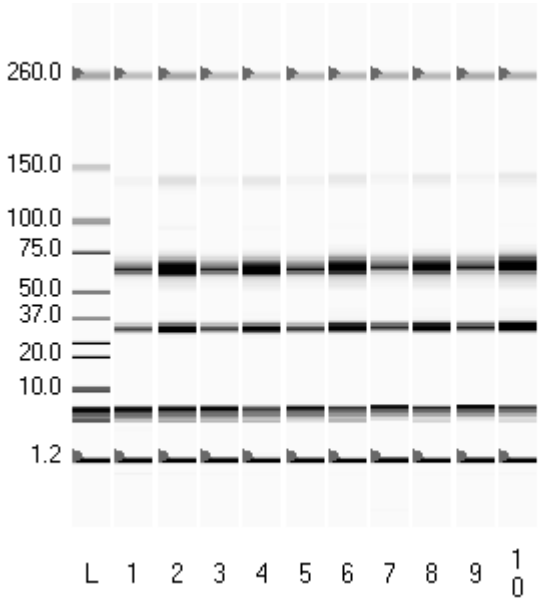


Fig. 2.2. Virtual gel generated by Test 2. L, Pro260 ladder; lanes 1,3,5,7, and 9, 500 ng/µl BGG; lanes 2,4,6,8, and 10, 1,000 ng/µl BGG. In this example, the virtual gel was "scaled to global".

2. Evaluate the separation of the Pro260 ladder. This is a critical step in data analysis because much of the automated data analysis performed by Experion software is based on the successful separation of the Pro260 ladder. To display the ladder electropherogram, click the ladder well in the project tree, or click on the lane labeled **L** in the virtual gel. The electropherogram should resemble the one shown in Figure 1.8.
3. Examine the separation of 1,000 ng/µl BGG sample (S1). Click on the sample name in the project tree or on the lane in the virtual gel. The electropherogram appears and should appear as shown in Figure 2.3. Under reducing conditions, BGG separates into light- and heavy-chain fragments; therefore, the electropherogram in Figure 2.3 has more peaks than does the electropherogram in Figure 1.9 (non-reducing conditions).

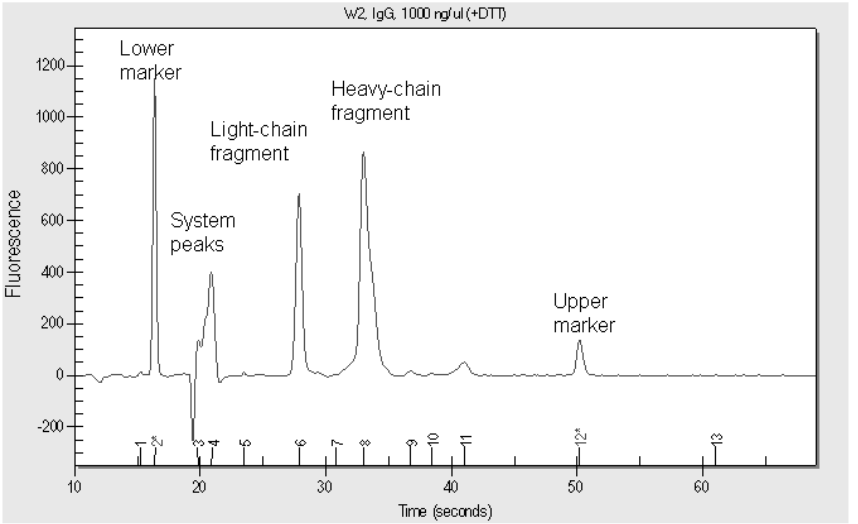


Fig. 2.3. Separation of the 1,000 ng/µl sample under reducing conditions. Under reducing conditions, BGG separates into light- and heavy-chain fragments.

4. Evaluate the percentage determination by Experion software (% Total calculation). Click on the **Results** tab, click on lane 2 in the virtual gel, and evaluate the values provided in the % **Total** column for each peak. For the sample shown in Figures 2.3 and 2.4, the light- and heavy-chain peaks correspond to peaks 6 and 8 and represent 34% and 62% of the total protein concentration, respectively.

Peak State	Peak Number	Mig. Time (secs)	Mol. Wt. (kDa)	Observation	% Total
	1	15.23			
	2	16.35	1.20	Lower Marker	
	3	19.84	6.20	System Peak	
	4	20.86	7.65	System Peak	
	5	23.48	13.32		0.2
	6	27.84	31.49		33.6
	7	30.75	47.95		0.1
	8	32.98	63.27		62.4
	9	36.72	94.51		0.7
	10	38.46	112.13		0.2
	11	41.03	138.90		2.8
	12	50.20	260.00	Upper Marker	
	13	61.01			

Light-chain fragment

Heavy-chain fragment

Fig. 2.4. Results table from Test 2 showing % Total (percentage determination) calculations. Results are shown for each of the peaks in the electropherogram shown in Figure 2.3.

5. Examine the % Total values for these peaks in both sample concentrations. Note that, regardless of the total sample concentration, the percentage of total represented remains the same.

Expected Performance for BGG in Test 2

Measurement	Typical Results
% Total (Percentage determination)	
Ligh-chain fragment	33–43% Total protein
Heavy-chain fragment	57–67% Total protein

If your results do not appear as they do in these examples, the most likely cause is insufficient reducing conditions in the sample buffer. Ensure that reducing buffer (“R”) was used to prepare samples and check that DTT was stored properly (at 4°C or reconstituted at –20°C).

Percentage determination is a particularly simple method of protein of quantitation because it does not rely on any internal standard. It is a highly reproducible qualitative method for estimating protein levels and purity, and it is a good method for tracking sample quality without relying on absolute concentrations.

Starter Kit Test 3 — Absolute Quantitation Using a Calibration Curve

3.1 Overview of Test 3

In this test, you create a calibration curve that you use to determine the concentration of a standard protein. You evaluate the linearity of the curve and check sizing and reproducibility. Experion™ software reports the results from relative and absolute quantitation methods side-by-side in the results table. The results of this test demonstrate how use of a calibration curve can significantly improve the accuracy of quantitation.

3.2 Assay Procedure

1. Set up the electrophoresis station, equilibrate the reagents to room temperature, and prepare the gel (G) and gel-stain solution (GS) as outlined in Sections 1.2.1–1.2.3. If the G and GS have already been prepared, equilibrate them as detailed in Section 1.2.2.
2. Prepare a serial dilution of the bovine γ-globulin (BGG, 2.0 mg/ml) supplied with the kit. Label six 0.65 ml microcentrifuge tubes 1–6. Tubes 1–5 are calibrants for creating the standard curve; tube 6 is the sample for analysis and quantitation (that is, your "unknown").

   Add 200 µl DEPC-treated water into each of the five calibrant tubes. Then add the following:

   Tube 1 (1,000 ng/µl): 200 µl BGG stock solution
   Tube 2 (500 ng/µl): 200 µl BGG dilution from tube 1
   Tube 3 (250 ng/µl): 200 µl BGG dilution from tube 2
   Tube 4 (125 ng/µl): 200 µl BGG dilution from tube 3
   Tube 5 (62 ng/µl): 200 µl BGG dilution from tube 4

   For tube 6 (400 ng/µl), combine 25 µl DEPC-treated water with 100 µl BGG dilution from tube 2.
3. Prepare both the reducing and nonreducing sample buffers ("R" and "NR") as described in Section 1.2.5. Prepare fresh sample buffer each day that an assay is run.
4. Prepare the samples and Pro260 ladder as described in Section 1.2.6. Use reducing buffer ("R") for preparing the Pro260 ladder L; use nonreducing sample buffer ("NR") for preparing samples S1–S6.
5. Prime and load the chip as described in Sections 1.2.7 and 1.2.8 and using the chip layout shown in Figure 3.1.

S6	S6	S6	GS
S5	S4	S3	GS
S2	S1	S6	GS
S6	L	GS	G

Fig. 3.1. Chip layout for Test 3. S1–S6, sample numbers; GS, gel-stain solution; G, Pro260 gel; L, Pro260 ladder.

6. Run the Pro260 analysis as described in Section 1.2.9. Enter the sample names and information appropriate to the chip layout for Test 3 (Figure 3.1).

3.3 Data Analysis

Evaluate the run and the analysis of the data by Experion software.

1. Evaluate the virtual gel to ensure that all lanes (samples) are visible. Check that the upper and lower markers are present in each sample (indicated by pink triangles) and that they are aligned across all lanes. Select **Graph > Scale to Global** to adjust the virtual gel so that it resembles an SDS-PAGE separation (Figure 3.2).

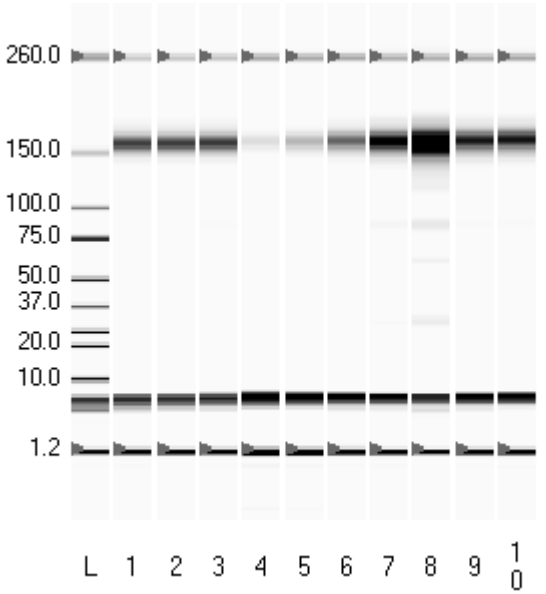
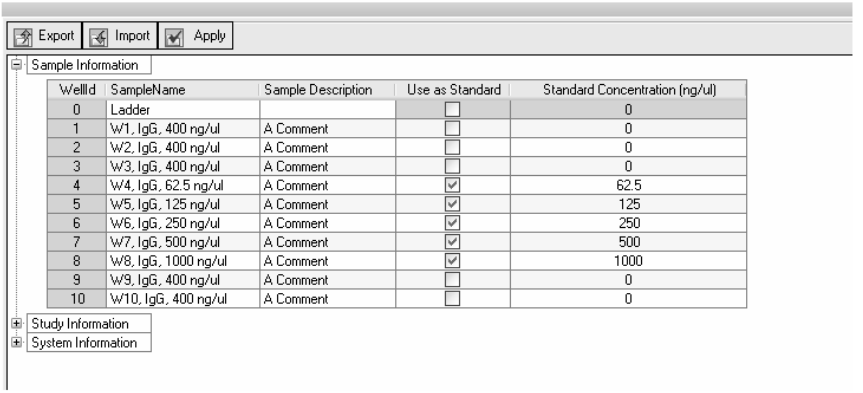


Fig. 3.2. Virtual gel generated by Test 3. L, Pro260 ladder; lanes 1–3, 9, and 10, BGG sample ("unknown"); lanes 4–8, BGG calibrants. In this example, the virtual gel was "scaled to global".

2. Evaluate the separation of the Pro260 ladder. This is a critical step in data analysis because much of the automated data analysis performed by Experion software is based on the successful separation of the Pro260 ladder. To display the ladder electropherogram, click the ladder well in the project tree, or click on the lane labeled **L** in the virtual gel. The electropherogram should resemble the one shown in Figure 1.8.
3. Examine the separation of the 1,000 ng/µl BGG sample (S1). Click on the sample name in the project tree or on the lane in the virtual gel. The electropherogram appears and should resemble the electropherogram shown in Figure 1.9.
4. Under the **Run Info** tab, designate the wells that contain the proteins for the calibration curve (wells 4–8 in the **Use as Standard** column) and enter their concentrations (Figure 3.3). Click **Apply**.



WellId	SampleName	Sample Description	Use as Standard	Standard Concentration (ng/ul)
0	Ladder		☐	0
1	W1, IgG, 400 ng/ul	A Comment	☐	0
2	W2, IgG, 400 ng/ul	A Comment	☐	0
3	W3, IgG, 400 ng/ul	A Comment	☐	0
4	W4, IgG, 62.5 ng/ul	A Comment	☑	62.5
5	W5, IgG, 125 ng/ul	A Comment	☑	125
6	W6, IgG, 250 ng/ul	A Comment	☑	250
7	W7, IgG, 500 ng/ul	A Comment	☑	500
8	W8, IgG, 1000 ng/ul	A Comment	☑	1000
9	W9, IgG, 400 ng/ul	A Comment	☐	0
10	W10, IgG, 400 ng/ul	A Comment	☐	0

Fig. 3.3. Designating calibrants in the Run Info tab.

5. Select **Analysis > Internal Std. and Std Protein Calibration Curve**. The **Internal Std. and Std Protein Calibration Curve** window appears.
6. Next to **Standard Protein Molecular Weight** (Figure 3.4), enter ***160*** (the region in which the software searches for a peak). Click **Apply** to update the calculation.

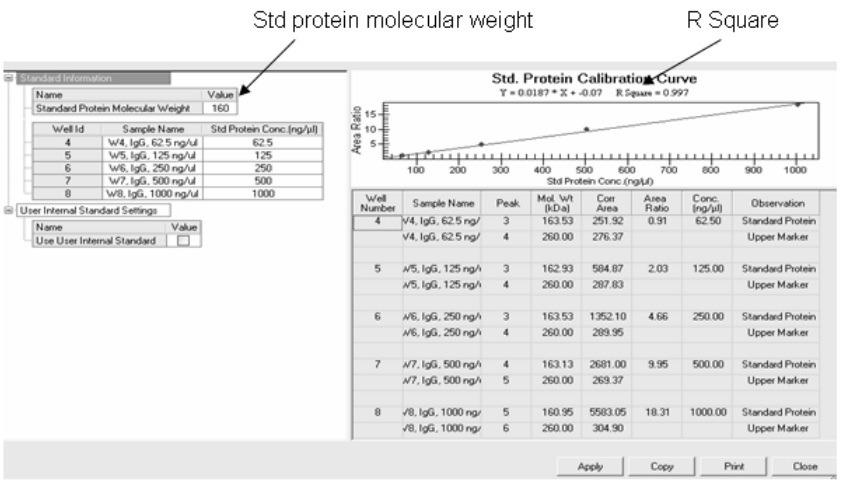
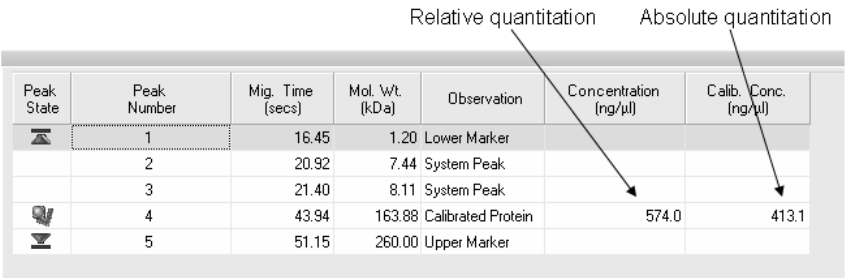


Fig. 3.4. Internal Std. and Std Protein Calibration Curve window.

7. The calibration curve appears and displays the statistical relevance of the regression. Evaluate the R Square value; it should be ≥0.97.

8. In the **Results** table, the concentration for each protein identified by Experion software is derived by relative quantitation against the 260 kD upper marker and appears in the **Concentration** column; the calibrated concentrations of proteins found in the size region specified appear in the **Calib. Conc**. column. The expected concentration for peak 4 (BGG) is 400 ng/µl. In the example in Figure 3.5, relative quantitation against the upper marker yielded a concentration of 574.0 ng/µl, while absolute quantitation yielded a concentration of 413.1 ng/µl.



Peak State	Peak Number	Mig. Time (secs)	Mol. Wt. (kDa)	Observation	Concentration (ng/µl)	Calib. Conc. (ng/µl)
	1	16.45	1.20	Lower Marker		
	2	20.92	7.44	System Peak		
	3	21.40	8.11	System Peak		
	4	43.94	163.88	Calibrated Protein	574.0	413.1
	5	51.15	260.00	Upper Marker		

Fig. 3.5. Results table from Test 3 showing relative quantitation (Concentration) and absolute quantitation (Calib. Conc.) calculations.

9. To evaluate the reproducibility of absolute quantitation, determine the %CV for wells 1–3, 9, and 10. Copy the data to Excel software and determine %CV as the standard deviation/mean x 100. Values should be ≤20%.
10. Calculate the mean concentration determined for the unknown samples. Note that the absolute quantitation method generates values that are more accurate (closer to the expected value of 400 ng/µl) than those derived by relative quantitation. Accuracy should be within 20% for 400 ng/µl BGG.

Expected Performance of BGG for Test 3

Measurement	Typical Results
Linear regression (calibration curve)	$r^2 \geq 0.97$
Absolute quantitation	
Reproducibility	%CV ≤ 20%
Accuracy	400 ± 80 ng/µl

Appendix A — How the Experion™ System Works

The Experion electrophoresis system performs electrophoresis of samples within a microfluidic chip. Within each chip, a series of microchannels connects the sample wells to a separation channel and buffer wells. A set of electrodes in the electrophoresis station applies a voltage across the microchannels, causing charged molecules in the samples to migrate into and through the separation channel. Samples are run sequentially, with a sufficient lag between them to prevent cross-contamination. For separation, the microchannels are filled with a proprietary gel-stain solution (GS) that acts as a sieving matrix; therefore, the sample fragments migrate through the separation channel at a rate based on their size and charge. Finally, sample fragments interact with a fluorescent dye during separation and are detected as they pass by a laser and a photodiode detector.

Protein analysis is accomplished with the Experion Pro260 analysis kit and involves the following steps:

1. **Preparing the chip (priming and loading)** — Priming fills the microchannels of the microfluidic chip with the gel-stain solution, which contains both the sieving matrix and fluorescent dye. Protein samples are then prepared in Pro260 sample buffer, which contains lithium dodecyl sulfate (LDS), and are added to the sample wells.
2. **Running the chip** — The chip is inserted into the electrophoresis station, and as the instrument lid is closed, electrodes come in contact with the solution in the wells. Voltage is applied to the sample wells of the chip, causing the charged, LDS-coated protein ions to migrate into the separation channel. In the separation channel, the different proteins separate as they move at different rates through the gel matrix, depending on their size. During separation, the fluorescent dye associates with the LDS micelles coating the proteins and with free micelles[9].
3. **Detecting the fragments** — As the fragments migrate towards the end of the separation channel, destaining occurs. Pro260 gel (G), which does not contain dye or LDS, flows alongside the sample. Diffusion of free LDS into this detergent-free zone reduces the concentration of LDS below its critical micellar concentration, releasing dye molecules from unbound micelles. When it is free of the hydrophobic interior of the LDS micelle, the dye fluoresces weakly; the background signal is thus reduced. Dye-micelle complexes are more stable to the destaining process when they are bound to proteins. Downstream from where destaining occurs, a laser beam excites the dye, causing it to fluoresce if it is bound to the LDS micelle-protein complexes. A photodiode detects the fluorescence, and Experion software plots the fluorescence intensity versus time to produce an electropherogram and a virtual gel image.
4. **Analyzing the data** — Following separation, Experion software subtracts background noise, removes spikes, identifies and integrates peaks, and assigns their sizes and concentrations. Following analysis, parameters may be changed and the data reanalyzed.

[9] An important difference between the Experion system and traditional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) lies in how sample fragments are detected: in SDS-PAGE, samples are generally stained in the gel once separation is completed, while in the Experion system proteins are stained with a fluorescent dye during separation.

How Experion Software Analyzes Proteins

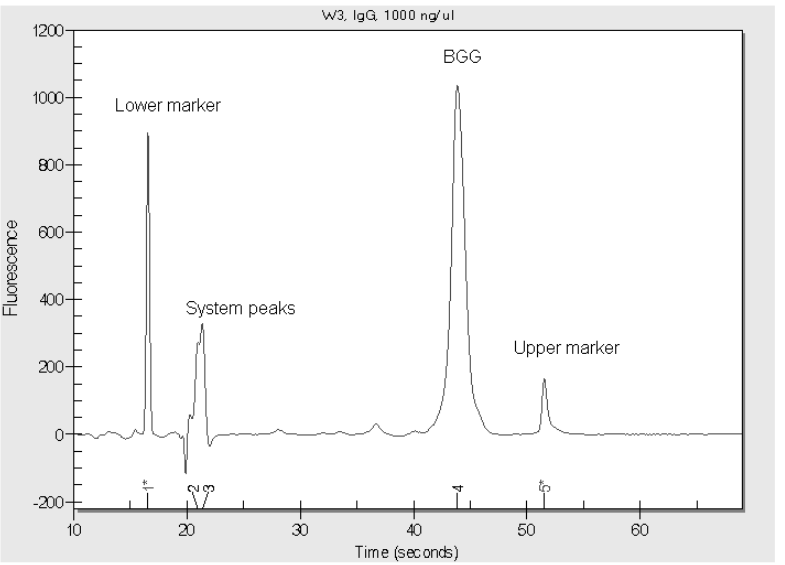
Data Presentation

As the photodiode detects fluorescent signals from the dye-protein-LDS complexes, Experion software converts the signal into an electropherogram (a plot of fluorescence vs. time). Experion Pro260 electropherograms generally have the following features:

Sample peak(s) — signal(s) generated by the sample protein(s)

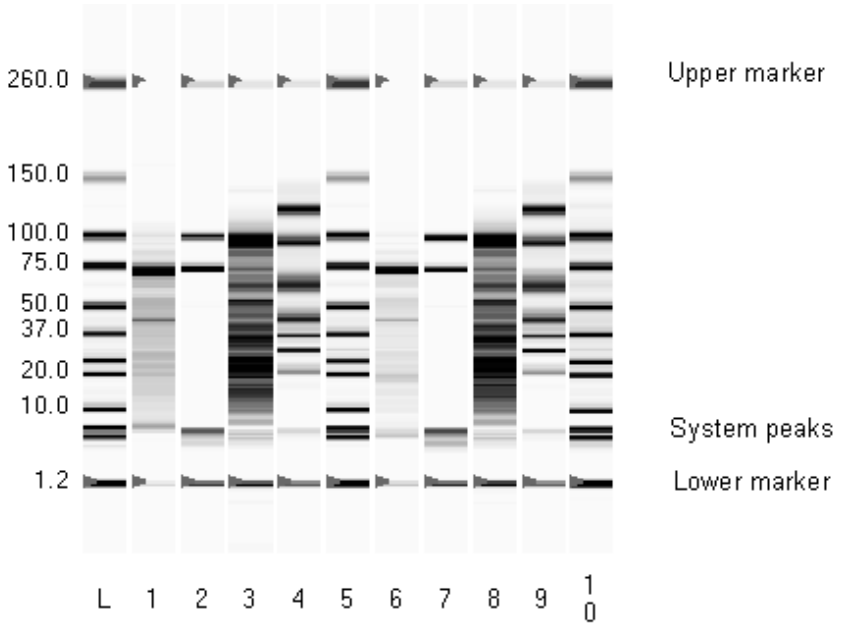
System peaks — cluster of signals generated by fluorescent detergent micelles. The system peaks are not considered in the quantitative concentration estimation

Upper and lower marker — signals generated by the internal upper (260 kD) and lower (1.2 kD) markers, which are included in the sample buffer and used to normalize the separation of proteins across all wells in the chips. The upper marker is also used as an internal standard for relative quantitation (see below). For each lane, comparison of the area of this peak with the area of every detected peak allows estimation of relative concentration



Electropherogram generated by Experion Pro260 analysis of bovine γ-globulin (BGG). The relative positions of the lower marker, system peaks, sample peak (BGG), and upper marker are shown.

Once separation occurs, these data are then converted into a densitometric, gel-like image, or virtual gel. Each lane in the virtual gel corresponds to a different sample. The sample and system peaks and upper and lower markers seen in the electropherogram also appear in the virtual gel.



Virtual gel generated by Experion Pro260 analysis. Shown are separations of the Pro260 ladder (L) and 10 samples on the same chip.

Once separation is complete, the software subtracts background noise, removes spikes, identifies and integrates peaks, and assigns their sizes and concentrations. Proteins are identified as peaks in the electropherograms and as bands in the virtual gel. The results of data analysis are also tabulated and presented in a Results table at the end of analysis. The types of data available include the following:

- Protein size (or mass, in kD)
- Protein concentration (in ng/µl) derived by relative and/or absolute quantitation
- % Of total sample (derived by percentage determination)

Peak State	Peak Number	Mig. Time (secs)	Mol. Wt. (kDa)	Corrected Area	Observation	Peak Height	Concentration (ng/µl)	% Total
	1	15.25		19.33		12.19		
	2	16.35	1.20	2379.57	Lower Marker	1114.31		
	3	18.60	4.42	21.54	System Peak	9.00		
	4	19.85	6.20	175.96	System Peak	120.40		
	5	20.90	7.71	881.96	System Peak	417.23		
	6	23.48	13.34	8.80		8.28	5.5	0.5
	7	27.89	31.72	620.16		302.03	388.0	35.8
	8	33.06	63.80	1105.62		354.22	691.8	63.7
	9	50.20	260.00	119.87	Upper Marker	105.20		

Sample Peaks Found 3
Total Concentration 1085.3 ng/µl

Results | Protein Search Result | Protein Search Criteria | Run Info | Settings

Results table generated by Experion Pro260 analysis.

Data Analysis: Normalization, Sizing, and Quantitation

Following separation, Experion software analyzes protein fragments using one or more of the following:

- Internal markers for normalizing the migration times of samples in different wells
- Pro260 ladder for determining fragment size (sizing)
- Internal markers or calibrants for determining concentration (quantitation)

Normalization: Aligning the Protein Peaks

To compensate for small variations in samples or factors influencing separation (pH, salt concentration, injection volumes, separation field, etc.), Experion Pro260 analysis uses two internal markers to normalize the migration times between samples. The two internal markers, an upper marker (260 kD) and lower marker (1.2 kD), are included in the Experion Pro260 sample buffer. Therefore, both of these markers are added to each sample and the Pro260 ladder. Inclusion of these markers and the normalization process ensures that the system software properly identifies peaks.

Protein Sizing

The first sample to be analyzed is the 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 purified, recombinant proteins of 10–260 kD. Experion software constructs a standard curve of migration time as a function of size from the Pro260 ladder separation. It then calculates the size of the proteins from the sample wells by comparing their migration times to the standard curve.

Protein Quantitation

Experion software offers two different types of protein quantitation methods: percentage determination and concentration determination. All protein quantitation measurements are based on the time-corrected peak area (corrected area) of each peak identified in an

electropherogram. The corrected area of a peak is proportional to the amount of the protein it represents in a mixture.

Percentage determination — measures the percentage of each protein in a protein mixture (% Total). This method is commonly used for determining protein content, protein purity, and protein stability

Concentration determination — provides the amount of the protein(s) in a protein mixture rather than just a percentage of the total. There are several different ways that concentration determination can be performed, and these methods provide more or less precision depending on the type of internal standard used and the extent to which a calibration curve is used

Relative quantitation — Experion software uses an internal standard to estimate the concentrations of sample proteins. Estimates are calculated by comparing the corrected peak area of the sample peak to that of the internal standard in each sample well. The internal standard can be either the upper marker (260 kD), which is included in the Pro260 sample buffer, or a user-defined protein (10–<260 kD) added to each sample at a known concentration.

Absolute quantitation — Experion software can also create a calibration curve to use for quantitation. To create the calibration curve, a defined protein calibrant at different concentrations is separated in different sample wells. The corrected peak areas of the different concentrations are compared to that of an internal standard (as with relative quantitation, the upper marker or a user-defined protein can be used as the internal standard). Experion software plots the resulting ratios as a function of protein concentration, and the sample protein concentration is then determined from this calibration curve.

Appendix B — Cleaning the Electrodes

Two types of cleaning procedures ensure the best results from the Experion™ electrophoresis station: 1) a deep-cleaning procedure, which applies electrode cleaner directly to electrode pins using swabs, and 2) a routine procedure, which only uses special cleaning chips and is performed after every run.

Method 1: Deep Cleaning

This manual procedure applies Experion electrode cleaner directly to the 16 platinum pins in the electrode manifold. Perform this procedure:

- Prior to first use of the Experion electrophoresis station
- Between a protein and an RNA analysis
- Any other time when RNase contamination is suspected or any contamination (for example, salt deposits or other precipitates) is visible on the electrodes
- Whenever a chip has been left in the instrument for an extended period of time (for example, overnight)

Perform this procedure with the electrode manifold in place (installed in the electrophoresis station). It requires the following supplies, all of which are included in the Experion Pro260 starter kit:

- Experion electrode cleaner
- Experion DEPC-treated water
- Foam swabs

Warnings: Keep the chip platform completely dry during this procedure. Cover the chip platform using plastic wrap and a paper towel to protect it from liquid.

1. Ensure that power to the electrophoresis station has been shut off.
2. Add 0.5–1 ml Experion electrode cleaner to a microcentrifuge tube. Insert a swab into the solution until it is saturated with the electrode cleaner.
3. Use the swab to lightly scrub each electrode pin one at a time, on all four sides. Do not press too hard on the pins, as they may bend. Finally, clean the tip of each pin. Add more electrode cleaner to the swab as necessary.

   -Or-

   Move the swab up and down, and side to side, 2–3 times along the columns and rows of pins. Finally, clean the tips of each of the pins. Add more electrode cleaner to the swab as necessary.
4. Repeat steps 2 and 3 twice, each time with a fresh swab soaked in DEPC-treated water.
5. Fill a cleaning chip with 800 µl DEPC-treated water, and place the chip in the electrophoresis station for 1 min. Then remove the chip.
6. Allow the pins to dry completely by leaving the lid open for at least 10 min.

Note: Refer to the Experion system instruction manual for more details and images.

Method 2: Routine Cleaning — Protein Analysis

Use this procedure to clean the electrode manifold in the Experion electrophoresis station immediately after each run.

Warning: Never store the cleaning chip inside the electrophoresis station. Store the empty cleaning chip covered to keep the wells clean.

1. Fill a cleaning chip with 800 µl DEPC-treated water[10]. 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 1 min.
4. Open the lid and remove the cleaning chip.
5. Allow the electrodes to dry for 1 min.
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.

[10] DEPC-treated water is not required for this cleaning application; however, always use ultrapure (at minimum 0.2 µm-filtered) water. Do not use autoclaved water.

Appendix C — First-Time Use of the Experion™ System

The first time that the Experion electrophoresis station is used, do the following:

1. Establish communication between the software and electrophoresis station.
   a. Power on the computer.
   b. Power on 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.
   c. Launch Experion software. Click the Experion software icon on the desktop or select **Start > All Programs > Bio-Rad Laboratories Experion > Experion**. If a prompt appears asking if Windows should block the program, click **No**.

   The following indicate that communication has been established:

   - A green dot with the last four digits of the instrument serial number appears in the lower right corner of the main software screen
   - The appearance of an electrophoresis station icon in the upper left corner

   When there is no connection, these indicators are absent and a "disconnected" message appears next to the **Start Run** button in the upper left corner of the screen. In addition, a grayed-out instrument icon appears in the upper left corner of the software screen.
2. Confirm that all power saver and/or power options are turned off before starting the assay (refer to the Experion system instruction manual for more details). Do not let the computer "sleep" during a run.
3. Clean the electrode manifold using Experion electrode cleaner and the deep cleaning procedure described in Appendix B.

Appendix D — Ordering Information

Catalog #	Description
700-7000	**Experion™ System**, 100–120/220–240 V, for protein analysis, includes electrophoresis station, priming station, software, USB2 cable, instructions (analysis kits sold separately)
700-7110	**Experion Pro260 Starter Kit**, includes 3 Experion Pro260 chips, 1 cleaning chip, Experion reagents, spin filters, IgG protein standard, DTT, cleaning swabs, electrode cleaner, tips and tubes to run 3 chips, instructions
700-7111	**Experion RNA StdSens Starter Kit**, includes 3 Experion RNA StdSens chips, 2 cleaning chips, Experion reagents, spin filters, total RNA standard, cleaning swabs, electrode cleaner, tips and tubes to run 3 chips, instructions
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
700-7256	**Experion Protein Ladder**, 60 µl
700-7251	**Experion Cleaning Chips**, 10
700-7254	**Experion Spin Filters**, 10
700-7252	**Experion Electrode Cleaner,** 250 ml
700-7253	**Experion DEPC-Treated Water**, 100 ml
163-2091	**ReadyPrep™ Proteomics Grade Water**, 500 ml
700-7264	**Swabs**, 25
500-0208	**Bovine Gamma Globulin (BGG) Standard Sample**, 2.0 mg/ml, 2 ml
VWR87001-688	**VWR barrier pipet tips, sterile** (narrow-bore, 20 µl universal pipet tips, RNase-free) (Not available from Bio-Rad. Purchase directly from VWR Scientific Products)
5395	**Experion Training Video: Chip Loading** (DVD) (also available at discover.bio-rad.com/Experion/)
10000975	**Experion Pro260 Analysis Kit Instruction Manual**
10001312	**Experion System Instruction Manual**

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