WESTERN BLOTTING

Western Blot Normalization Using Image Lab™ Software

Quick Start Guide

Total Protein Normalization Using Stain-Free Gels

This guide describes the steps to normalize your chemiluminescent blot with stain-free technology.

1 Image your stain-free blot*

- Click **New Protocol**
- In Application box, select **Blots** then **Stain-Free Blot**
- In Imaging Area box, select gel/blot size. For mini- or midi-sized blots, select **Bio-Rad Ready Gel®** or **Bio-Rad Criterion™ Gel**
- Click the **Position Gel** button. Center stain-free blot on imaging plate
- Click **Run Protocol**

* Instructions assume that the stain-free gel was activated prior to the electrophoretic transfer. For best results select 1 min activation for stain-free gels.

2 Image your chemiluminescent blot

- Add Clarity™ western ECL substrate to the centered blot from step 1. Incubate 5 min before imaging
- Click **New Protocol**
- Select **Blots** then choose appropriate chemi setting
- Use same image area as the stain-free blot
- Select exposure setting **Auto Optimization** for **Intense Bands**
- Click **Position Gel** button to confirm blot is centered
- Click **Run Protocol**

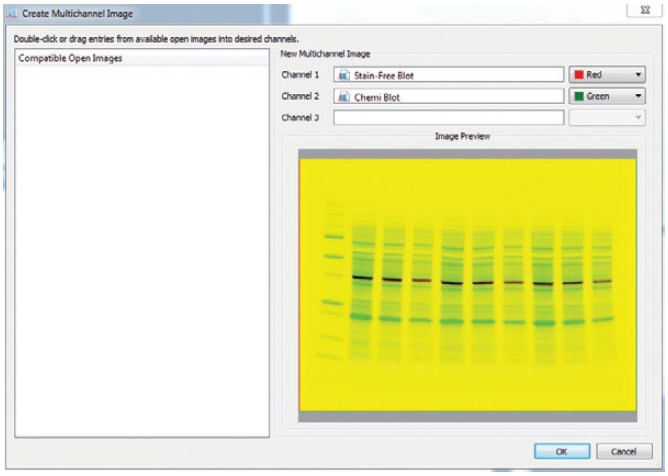

Fig. 1. Image Lab software setting for linking stain-free and chemiluminescent blot images.

3 Creating a multichannel image

- With the stain-free and chemiluminescent blot images opened, select **File** and **Create Multichannel Image** (Figure 1)
- Drag stain-free blot image to Channel 1. Drag chemiluminescent blot image to Channel 2. Click **OK**
- Click RGB icon to deselect overlaid view. Remaining channels will be the stain-free blot and chemiluminescent blot as shown in Figure 2

Fig. 2. Multichannel image of stain-free and chemiluminescent blot images.

4 Normalize your western blot

- Detect the lanes of the stain-free blot by highlighting the stain free blot channel (Figure 3). Click **Lane and Bands** from the Analysis Tool Box; in the Lane Finder box, click **Automatic**
- Adjust the lanes of stain-free blot by using the lane adjustment tools provided to optimize the lane sizes, shapes, and boundaries

  Tips:
  - For accurate quantitation adjust the lanes to include the entire width of all bands
  - Lanes should be the same size and span the same region without touching adjacent lanes

- Adjust background subtraction of the lanes by setting disk size to 70 (Figure 4)

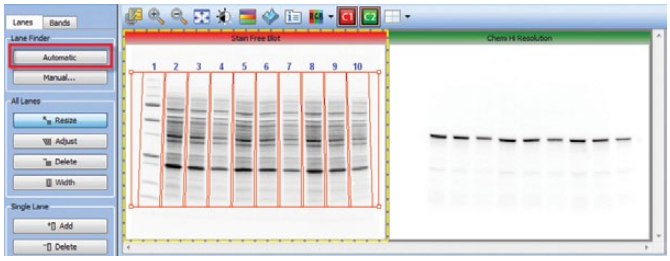

Fig. 3. Automatic detection of the lanes of the stain-free blot.

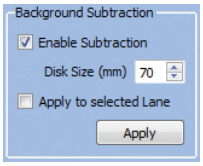

Fig. 4. Lane background subtraction.

- Copy (Ctrl + C) the lanes established from the stain-free blot and paste (Ctrl + V) to the chemiluminescent blot (Figure 5). Adjust lanes on the chemiluminescent blot, if needed
- With chemiluminescent blot highlighted in yellow, detect the bands in chemiluminescent blot by selecting **Bands** tab and then **Detect Bands** (Figure 6). Select appropriate detection sensitivity setting and click **Detect**

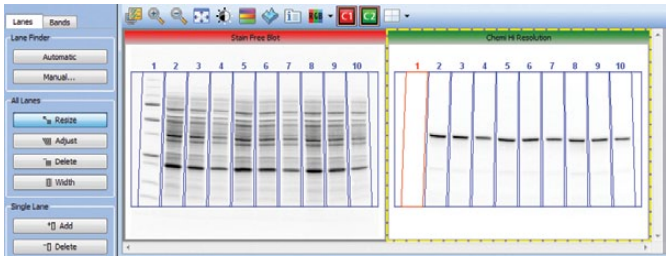

Fig. 5. Lane detection of the chemiluminescent blot.

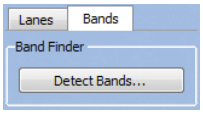

Fig. 6. Band detection of the chemiluminescent blot.

- Return to Analysis Tool Box and select **Normalization**. Assign stain-free blot as normalization channel (Figure 7). The **Total Lane Protein** radio button must be selected (default setting)
- Return to Analysis Tool Box and select **MW Protein Standard**. In the stain-free blot image check the box below all MW standard lanes (Figure 8)

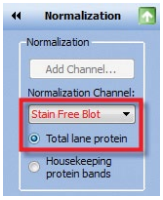

Fig. 7. Stain-free blot as the normalization channel.

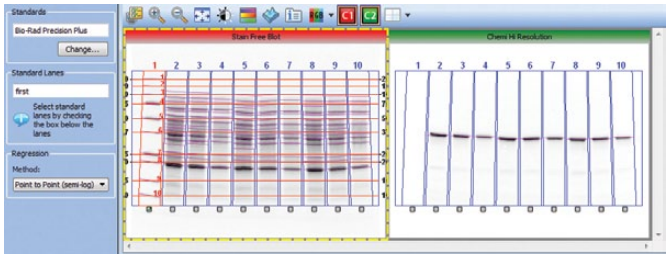

Fig. 8. Selecting the molecular weight lane in the stain-free blot image.

- View the normalized volumes by selecting **Analysis Table** from the main toolbar (Figure 9). All calculations will be performed by the software, including the normalization factor and normalized volumes. The chemiluminescent blot channel intensity values are now adjusted for variation in the protein loading between different lanes. This will allow accurate comparisons of target protein intensities across all lanes of a gel

  Note: The software will automatically select the first nonstandard lane as the reference lane against which all other lanes are compared.

$$\text{Normalization factor} = \frac{\text{total volume (Intensity) stain-free reference lane}}{\text{total lane stain-free volume (Intensity) of each lane}}$$

$$\text{Normalized volume} = \text{normalization factor} \times \text{volume (Intensity)}$$

- From the Analysis Table tools, click **Display Data Options** to customize the data table (Figure 10)
- From the Analysis Table tools, click **Export Analysis Table to Excel** for additional analysis (Figure 11)

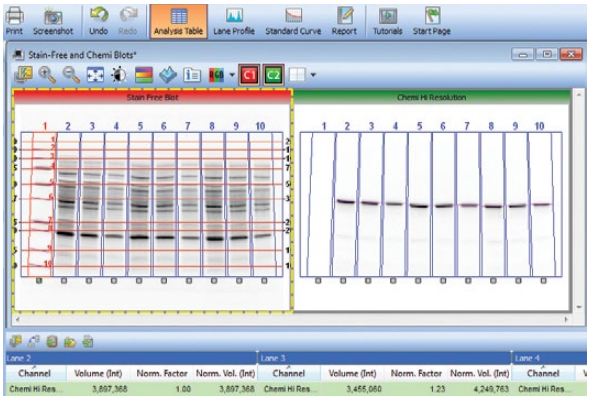

Fig. 9. Analysis table with the calculated normalization factor and normalized volumes.

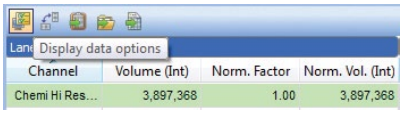

Fig. 10. Customize the data table.

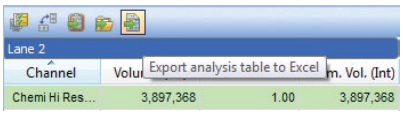

Fig. 11. Export the data to Excel.

- Once exported to Excel, the results can be arranged as shown in Table 1

Table 1. Intensity values for protein identified in the chemiluminescent blot

Channel	Lane Number	Band Number	Volume (Intensity)	Normalization Factor	Normalized Volume (Intensity)
Chemi	Lane 2	1	3860064	1	3860064
Chemi	Lane 3	1	3406560	1.29	4416034
Chemi	Lane 4	1	2331168	1.89	4408112
Chemi	Lane 5	1	3782112	1.05	3981556
Chemi	Lane 6	1	3383328	1.31	4445670
Chemi	Lane 7	1	2444832	1.97	4822224
Chemi	Lane 8	1	3445536	1.09	3739923
Chemi	Lane 9	1	2851872	1.38	3934866
Chemi	Lane 10	1	1940544	2.03	3937656

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin 6434 Rev A US/EG *13-1227 0613 Sig 1212*