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 **Position Gel**. 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** to confirm blot is centered
- Click **Run Protocol**

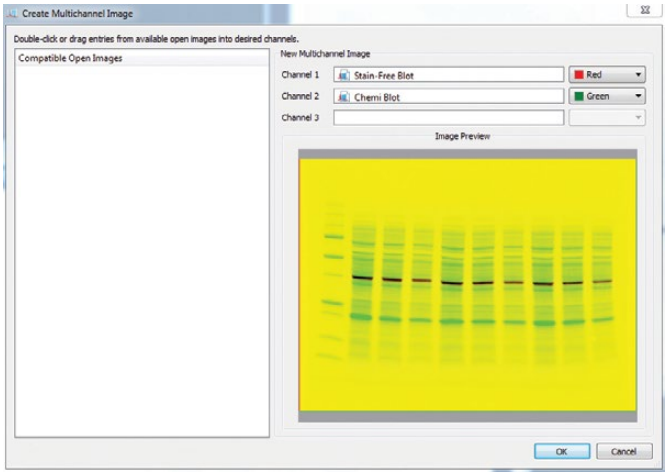

Fig. 1. Image Lab Software setting for linking stain-free and chemiluminescent blot images.

3 Create 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 the RGB icon to deselect the overlaid view. Remaining channels will be the stain-free blot and chemiluminescent blot as shown in Figure 2

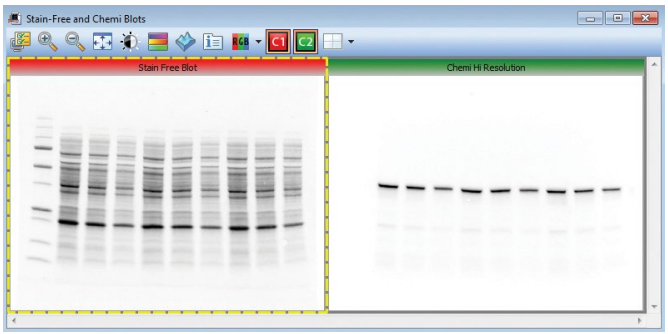

Fig. 2. Multichannel image of stain-free and chemiluminescent blot images.

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 the 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

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

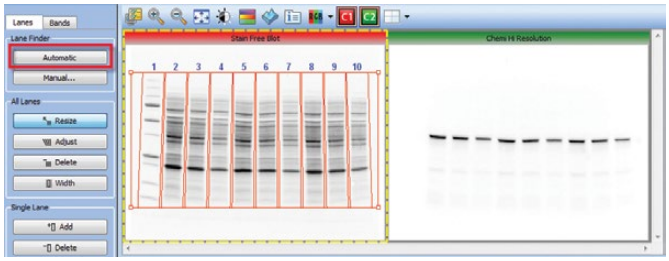

Fig. 3. Automatic detection of the lanes of the stain-free blot.

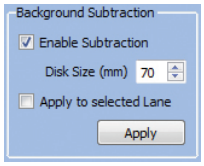

Fig. 4. Lane background subtraction.

- Click **Resize** under All Lanes to select all lane profiles
- Copy (PC: Ctrl-C; Mac: Command-C) the lanes established from the stain-free blot and paste (PC: Ctrl-V; Mac: Command-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 the chemiluminescent blot by selecting the **Bands** tab and then **Detect Bands** (Figure 6). Select the appropriate detection sensitivity setting and click **Detect**

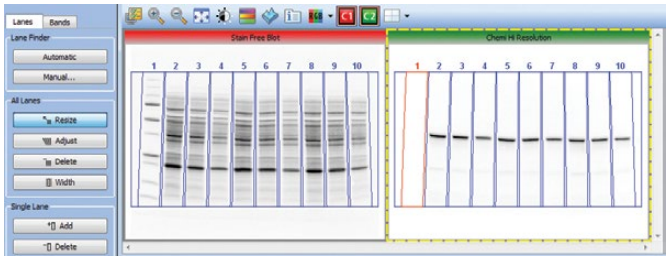

Fig. 5. Lane detection of the chemiluminescent blot.

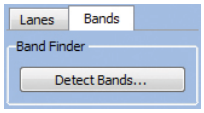

Fig. 6. Band detection of the chemiluminescent blot.

- Click **Lane Profile** to check that the lane profiles are consistent across the lanes
- Return to the Analysis Tool Box and select **Normalization**. Assign **Stain Free Blot** as the normalization channel (Figure 7). Ensure the **Total Lane Protein** radio button is selected (default setting)
- Return to the Analysis Tool Box and select **MW Protein Standard**. In the stain-free blot image check the box below all MW standard lanes (Figure 8)

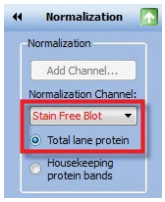

Fig. 7. Stain-free blot as the normalization channel.

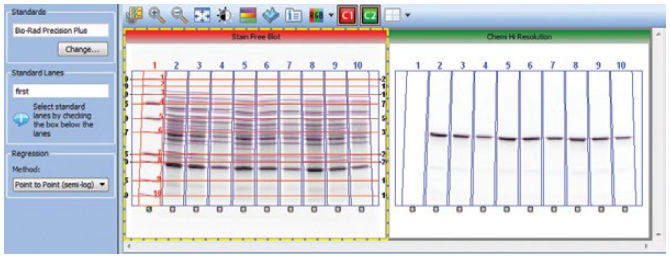

Fig. 8. Selecting the molecular weight lane in the stain-free blot image.

- 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.

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

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

- 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)

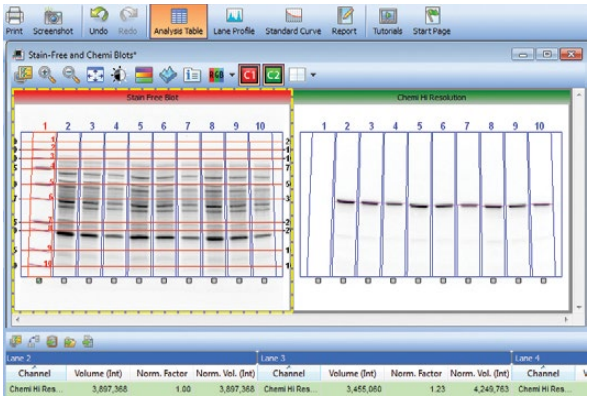

Fig. 9. Analysis table with the calculated normalization factor and normalized volumes.

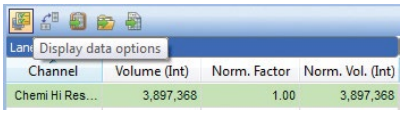

Fig. 10. Customize the data table.

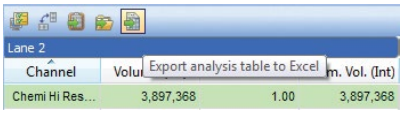

Fig. 11. Export the data to Excel.

- 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	3,860,064	1	3,860,064
Chemi	Lane 3	1	3,406,560	1.29	4,416,034
Chemi	Lane 4	1	2,331,168	1.89	4,408,112
Chemi	Lane 5	1	3,782,112	1.05	3,981,556
Chemi	Lane 6	1	3,383,328	1.31	4,445,670
Chemi	Lane 7	1	2,444,832	1.97	4,822,224
Chemi	Lane 8	1	3,445,536	1.09	3,739,923
Chemi	Lane 9	1	2,851,872	1.38	3,934,866
Chemi	Lane 10	1	1,940,544	2.03	3,937,656

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Bulletin 6434 Ver B US/EG *15-1104 0915 Sig 1214*