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 Create a multichannel image and normalize your western blot

- With the stain-free and chemiluminescent blot images open, select **Lane and Bands** (Figure 1)
- Select **Add channel** in the Normalization Channel dropdown menu (Figure 2)
- Drag the stain-free blot to the Normalization Data channel and click **OK** (Figure 3)

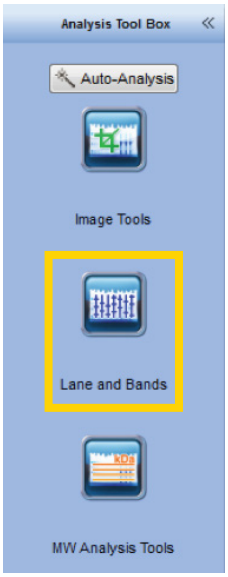

Fig. 1. Normalization is found under Lane and Bands in the Analysis Tool Box.

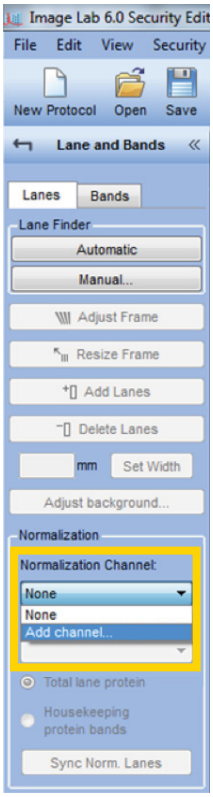

Fig. 2. Add normalization channel.

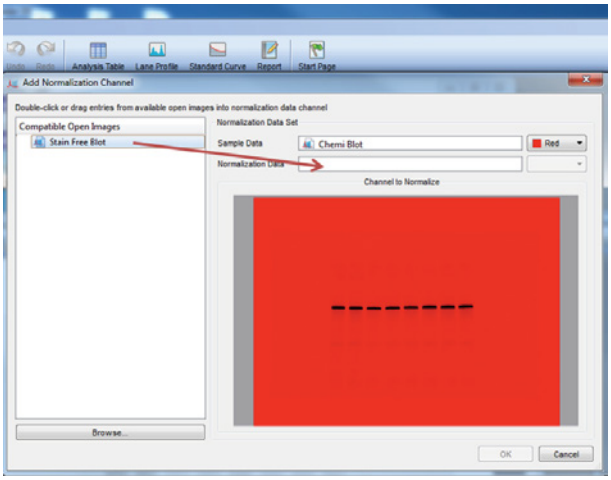

Fig. 3. Create a multichannel image of the stain-free and chemiluminescent blot images.

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- Select the appropriate band detection settings based on blot band intensity (Figure 4). Image Lab Software will automatically detect lanes and bands
- If needed adjust the lane frame by using the **Adjust Frame** and **Resize Frame** buttons (Figure 5). Adjust the individual lanes by selecting the lane that needs adjustment and moving the anchor points (Figure 5)

Note: When lanes are manually moved or adjusted, previously detected bands are removed for any lanes that are adjusted. Go to the Bands tab, click **Detect Bands...**, then select the previous appropriate band detection settings to add these bands again.

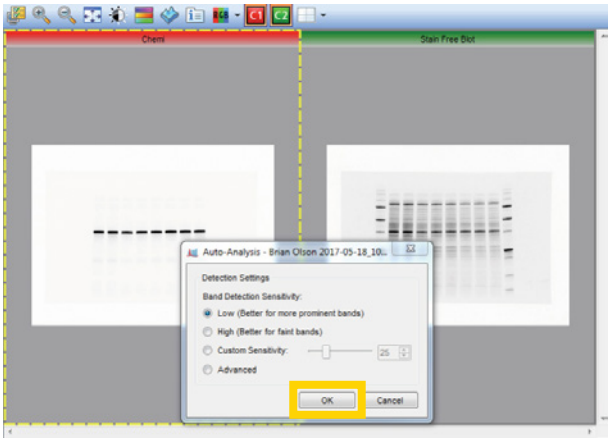

Fig. 4. Automatic lane and band detection.

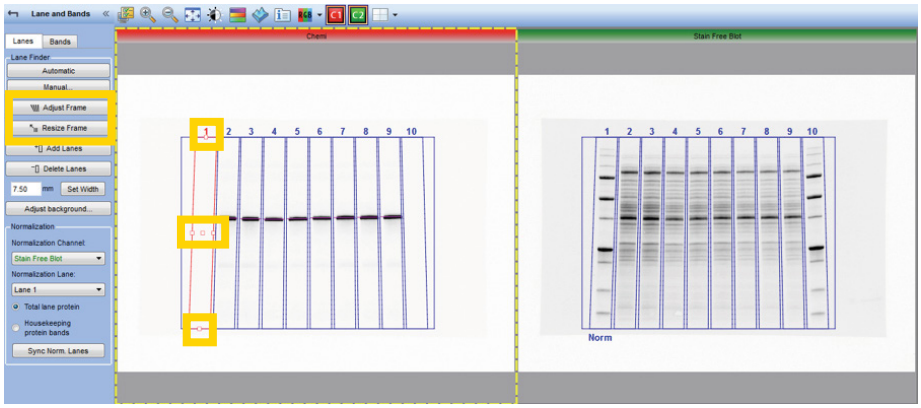

Fig. 5. Manual lane adjustment.

- In the Normalization Lane dropdown menu, select a lane to use for normalization.
  Do not use a lane with a protein standard as the normalization lane (Figure 6)

Note: The sample in the normalization lane is typically the null or wild-type condition in an experiment and is considered to be 1x or onefold. All lanes will be compared to this null condition. True protein normalization will happen when comparing the chemiluminescence signal in each lane to its corresponding stain-free lane.

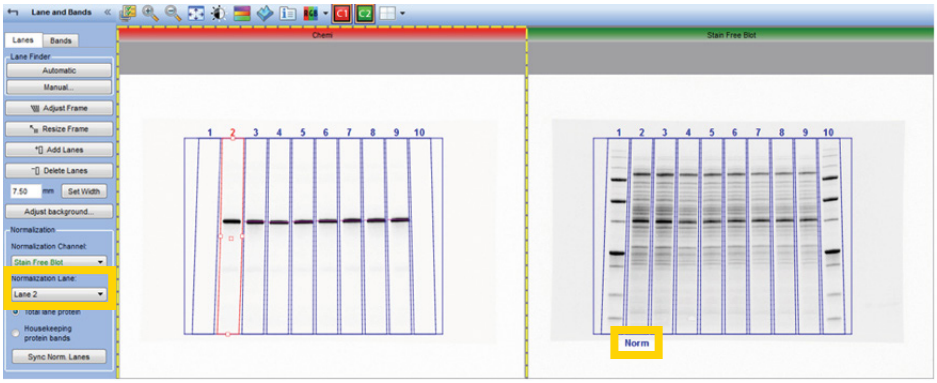

Fig. 6. Select normalization lane.

2 View analysis results

- View the normalized volumes by selecting **Analysis Table** from the main toolbar (Figure 7). 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
- From the Analysis Table tools, click **Display data options** to customize the data table (Figure 8)
- From the Analysis Table tools, click **Export analysis table to Excel** for additional analysis (Figure 9)

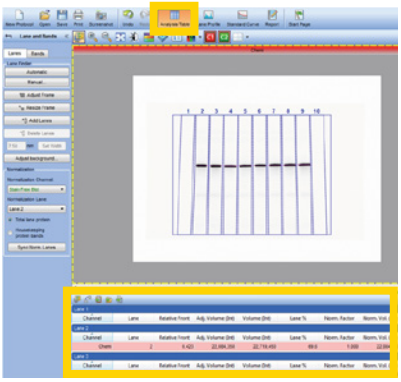

Fig. 7. Analysis table with the calculated normalization factor and normalized volumes.

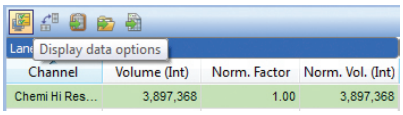

Fig. 8. Customize the data table.

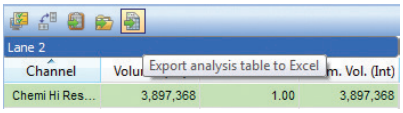

Fig. 9. Export the data to Excel.

- 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

Note: The Normalized Volume (intensity) value represents each chemiluminescent signal (band) that is properly total protein normalized to the stain-free signal in its corresponding lane. This is the value you should use for further analysis and, ultimately, publication of experimental results.

Visit **bio-rad.com/web/ImageLabNormalization** for more information.

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