Blot imaging on Jess using the blot cartridge

Quick Reference Guide

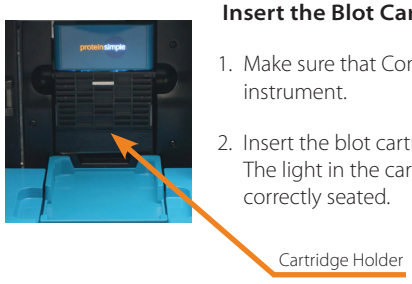
STEP 1 - GET READY

Prepare the membrane and assemble the Blot Cartridge

Using traditional Western blot procedures, your proteins should have been transferred from an SDS-PAGE gel to either a nitrocellulose or PVDF blotting membrane. Take care to handle the membrane by its edges only.

1. Cut membrane to appropriate size that will fit within the laser etched area on the blot cartridge glass.
2. Prepare 1.5 mL of your preferred enhanced chemiluminescence (ECL) substrate.
3. Pipette 750 µL of ECL onto the black backing surface along the top of the blot cartridge.
4. Gently lay the membrane protein side up onto the black backing surface, beginning from the top and working down to minimize bubble formation.
5. Ensure the area of interest (3" x 2") is placed within the boundaries of the lines laser etched onto the glass cover.
6. Pipette 750 µL of ECL along the top edge of the laid membrane.
7. Close the glass cover of the blot cartridge gently to avoid producing bubbles.
8. Wipe excess liquid from the bottom of the cartridge.

Steps for assembling a blot cartridge.



Insert the Blot Cartridge

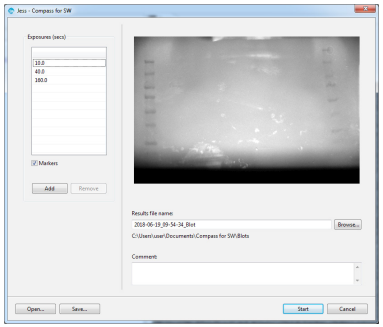
1. Make sure that Compass for Simple Western software is connected to the instrument.
2. Insert the blot cartridge containing the membrane into the Cartridge Holder. The light in the cartridge holder will change from orange to blue when correctly seated.

The software will automatically change to the Blot screen:

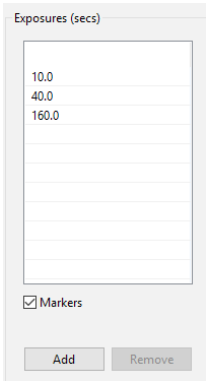
NOTE: Jess's door must be closed before starting the imaging run.

STEP 2 – START THE IMAGING RUN

Once the blot cartridge is installed and Jess's door is closed, Compass will automatically provide a preview image.

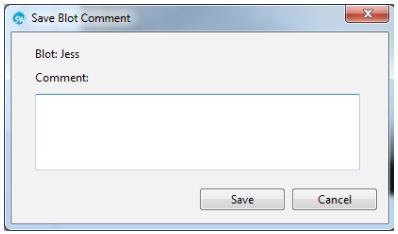


1. Edit the exposure settings in the **Exposures** table:



a. To add exposure: Click **Add** then select the exposure time to change it.
b. To remove exposure: Select the exposure and click **Remove**.
c. To edit exposure time: Click on an exposure and enter a new time.
d. Check the **Markers** box if the blot contains a molecular weight ladder.

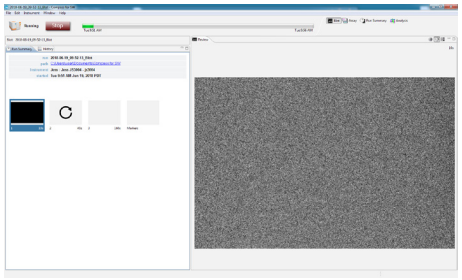
3. To load the saved settings from an existing blot imaging protocol file (*blot), click **Open**. Select the blot imaging protocol file and click **Open**.
4. You can change the **Results File** name and location if desired. Blot image file names are automatically generated as "date_time_blot" and saved in the Compass for SW\Blots folder of your My Documents folder.
5 Add comments that you would like saved with your Blot run in the Comments box (optional).



6. To save the settings associated with this blot imaging protocol, click **Save** to bring up a Save dialog (optional)
7. Press **Start** when ready.

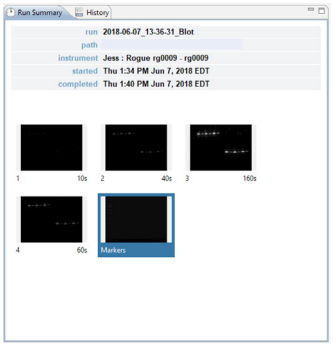
STEP 3 – IMAGING RUN SUMMARY AND ANALYSIS

1. When the imaging run starts, thumbnails are shown in the **Run Summary** screen.



As images are acquired, they populate the predefined thumbnail boxes and will be displayed in the **Review** pane. Unless you click on a thumbnail, the most recently acquired image displays in the **Review** pane. Images that haven't been taken yet will display as blank until the image is acquired.

2. The **Run Summary** pane will display thumbnail images for each exposure configured in the imaging protocol:



NOTE: The **Markers** image is taken at the end of the blot imaging run. If you click **Stop** before all exposures are taken, the software will prompt you to confirm that you want to stop the run before the **Markers** image is taken.

3. You can adjust and enhance the blot image using the **Review** pane toolbar, to the right of the screen.

Buttons in the **Review** pane toolbar:

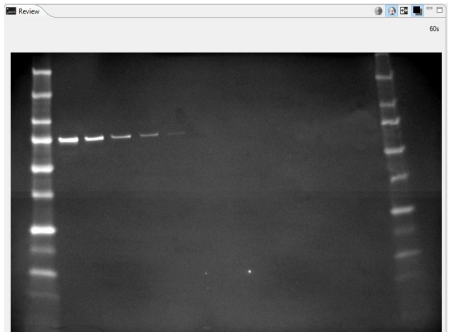

Contrast Adjustment


Auto Contrast


Invert

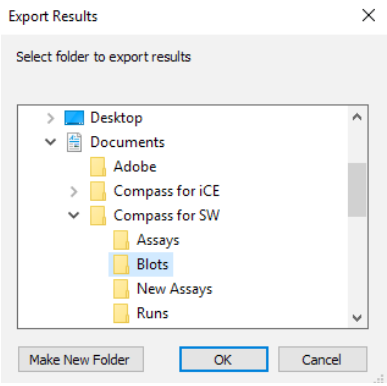

Overlay markers

Blot image **Review** data is shown in the following example:



Exporting Image Files

To export raw images for further analysis, from the main **File** menu, select **Export Images**. Select a folder to export the image files to.



Once export is complete, Compass will automatically launch a Windows Explorer window for you to access the saved files. Images are saved in .png format. Compass for SW will save raw and adjusted images to the chosen folder.

Raw chemiluminescence images are saved as <Results File Name>_Chemi_<Exposure Number>_<Exposure Time>.

Adjusted images, as shown in the **Review** pane, are saved as <Results File Name>_<Exposure Time>_ view.

Raw and processed marker images are saved as <Results File Name>_Markers and <Results File Name>_Markers_view, if selected during the blot imaging protocol.

STEP 4 – POST-IMAGING PROCEDURES

When imaging is done, you'll need to:

1. Remove the blot cartridge.
2. Remove your membrane and clean the blot cartridge by rinsing it with water. Dry with a lint-free wipe.
3. Wipe down the area underneath the installed blot cartridge within the instrument using a dry, lint-free wipe.
4. Keep the blot cartridge in a dry, clean area protected from dust.

Recommended Molecular Weight Ladders

The following molecular weight protein ladders are recommended to be used with the electrophoresis gels:

Ladder Name	Ladder Vendor and Part Number	Recommended Volume
PageRuler™ Prestained NIR Protein Ladder	Thermo Scientific, 26635	2 - 5 µL
Spectra™ Multicolor Broad Range Protein Ladder	Thermo Scientific, 26634	5 - 10 µL
Precision Plus Protein™ All Blue Prestained Protein Standards	Bio-Rad, 1610373	2 - 5 µL

Note: Ladders were tested using Bio-Rad 4–20% Mini-PROTEAN® TGX Stain-Free™ Protein Gels, 15 well, 15 µL, part number 4568096