Cell Biosciences™

Applying Molecular Weight Markers to Chemiluminescent blots in AlphaView® Q Software

Applies to AlphaView Q version 2.0 and above.

Introduction

AlphaView Q is an easy to use software analysis package designed for the quantitative analysis of fluorescent and chemiluminescent Western blots. This note describes the Annotate module within AlphaView Q software and details how to denote molecular weight markers on a chemiluminescent Western blot.

Adding Molecular Weight Markers Using the Annotate Module

The Annotate module provides the image annotation tools needed to specify molecular weight markers on a Western blot (Figure 1). The Annotate module is found within the Enhancement Tool tab in AlphaView Q software (Figure 2).
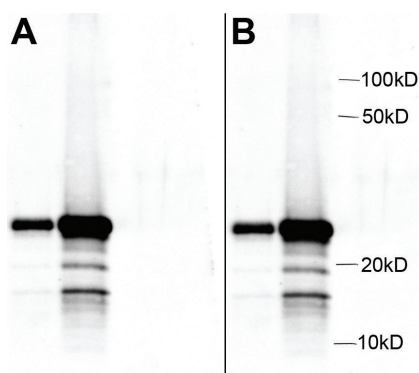

Figure 1. Sample chemiluminescent Western blot. (A) shows the sample blot without annotations specifying the molecular weight marker. (B) shows the sample blot after overlay with the molecular weight marker annotations is applied.
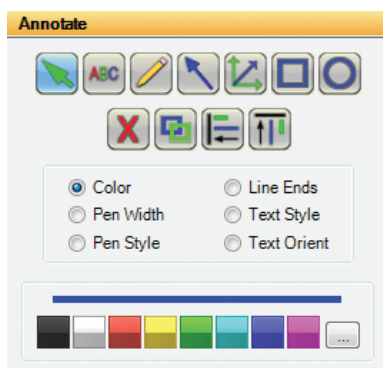

Figure 2. The Annotate module.

1. Acquire Image. The first step is to acquire your chemiluminescent image. Make sure the aperture is set to the lowest F stop value, that the filter position is set to "chemiluminescence" (no filter), and the lights are off in the cabinet. Once the image is captured, save your image by choosing File, Save As. Do not change the zoom or focus at this time.

2. Acquire White Light Image. Next, acquire a second image, this time using reflective white light to visualize the blot. If the image appears too bright, close the aperture by increasing the F stop value of the lens, without changing the zoom or focus.

It is important to use the same resolution/speed setting for acquiring the white light image. If necessary, use the EtBr filter to gain enough contrast in the image to visualize colorimetric markers.

3. Draw Annotations. Once you have acquired the white light image of the blot, open the Annotate module (Figure 2). Beginning with the pencil tool, use the mouse pointer to draw lines to specify the position of molecular weight markers on the white light image of your blot (Figure 3).

> Tip: You can customize the color, width and style of the lines using the Color, Pen Width and Pen Style Menus. For best results, we recommend using white or black text for the overlay.
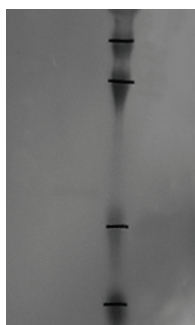

Figure 3. Sample blot image taken with reflective white light. After the image was captured, the pencil tool was used to draw an overlay with 4 lines, specifying molecular weight markers.

Molecular weight values can now be added to your overlay using the text feature. Choose the "ABC" selection and type the molecular weight that corresponds to each line drawn (Figure 4).

> Tip: To change the font, font size or the orientation of the text, use the Text Style and Text Orient Menus.
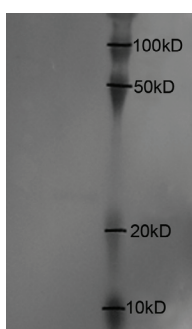

Figure 4. Sample Western blot overlay with molecular weight markers specified using the pencil tool and text feature in the Annotate module.

Save the overlay by selecting the Overlay menu at the top of the screen and the Save Overlay option (Figure 5). The overlay will be saved as an .ovr file.
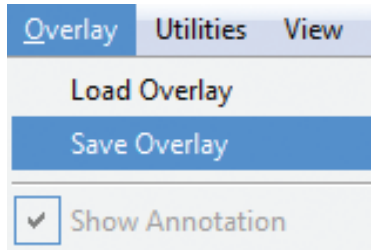

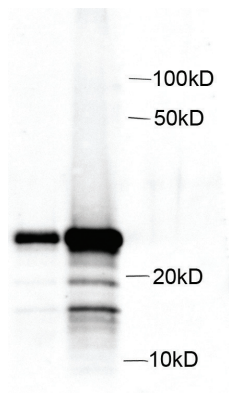**Figure 5.** Overlay menu with Save Overlay selected.

Figure 6. Sample Western blot with overlay applied.

4. Applying Overlay. Now that you have created and saved your overlay it is time to apply it to your original image. Open the original Western blot image; it should be at the top of the "Recent Files" list under the File menu. Once loaded, go to the Overlay menu at the top of the screen and select Load Overlay. Choose the overlay for the blot; it should have an .ovr file extension. The overlay will automatically be applied to your image (Figure 6).

5. Saving Image with Overlay. To save the image with the overlay applied, choose File and Save Modified. The saved image can be converted to an 8 bit image for export into other programs by selecting "Conversion" from the Image Menu and selecting "8 bit".

AlphaView Q is an easy-to-use software tool for both image acquisition and analysis of multicolor fluorescent Western blots and Chemiluminescent Western blots. AlphaView Q is specifically designed to meet the requirements for multichannel image analysis in molecular biology labs by providing tools in the software for loading control normalization. AlphaView Q comes standard with the FluorChem Q, a Western blot imaging system for fluorescent and chemiluminescent blots.

For more information, visit us at: http://www.cellbiosciences.com