iBright™ Imaging Systems Sample Blot

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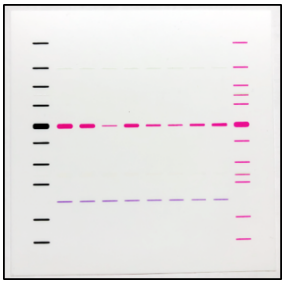


For demonstration of:

- Chemiluminescent acquisition
- Fluorescent acquisition
- Molecular weight determination
- Normalization

Image acquisition

Chemiluminescent Blot Mode

1. Place sample blot in the center of the imaging tray.
2. Select **Smart Exposure** to get an estimated acquisition time and a preview of how the signal will look at the recommended time.
3. If needed, adjust the exposure time. Select **Capture** to acquire the image.

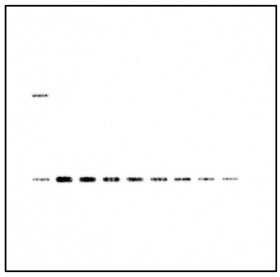


Fig. 1 Chemiluminescent Image

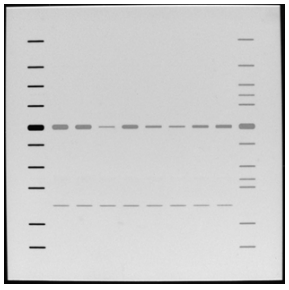


Fig. 2 Membrane

Fluorescent Blot Mode

1. Place the sample blot in the center of the imaging tray.
2. Assign a dye for each channel you want to capture. For the sample blot, select any or all of the following dyes:
   - Alexa Fluor™ 488 (Ex. 455-485 nm; Em. 515-564 nm, green bands)
   - Alexa Fluor™ 555 (Ex. 515-545 nm; Em. 568-617 nm, purple and pink bands)
   - Alexa Fluor™ 680 (Ex. 610-660 nm; Em. 675-720 nm, pink bands)
3. Select **Smart Exposure** to get an estimated acquisition time and a preview of how the signal will look at the recommended time.
4. If needed, adjust the exposure time. Select **Capture** to acquire the image.

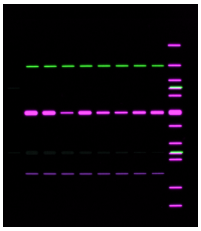


Fig. 3 Composite

Fig. 4 Alexa Fluor™ 488

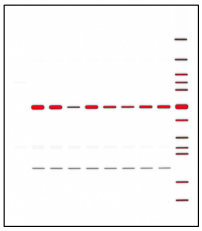


Fig. 5 Alexa Fluor™ 555

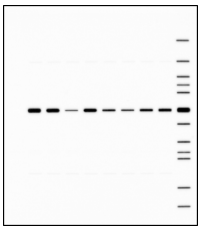


Fig. 6 Alexa Fluor™ 680

Analysis

After capturing an image of the Sample Blot:

1. Select the **Analyze** icon under the image viewport. The software will automatically identify analysis frame(s), lanes, and bands.
2. Under **More Options**, select the **Add Marker** button.
   a. Select the lane that contains the molecular weight ladder desired for calibration by touching + above the desired lane. Lane 1 or Lane 12.
   b. Use the menu on the right side of the screen to indicate the appropriate molecular weight ladder. Lanes 1 and 12 both contain the iBright™ Prestained Protein Ladder. In Lane 1, only 10 of 12 bands are visible; you need to delete the 80 kDa and 30 kDa bands from the marker list in order to get an accurate MW calculation. In Lane 12, all bands are identified in the Alexa Fluor™ 680 channel.

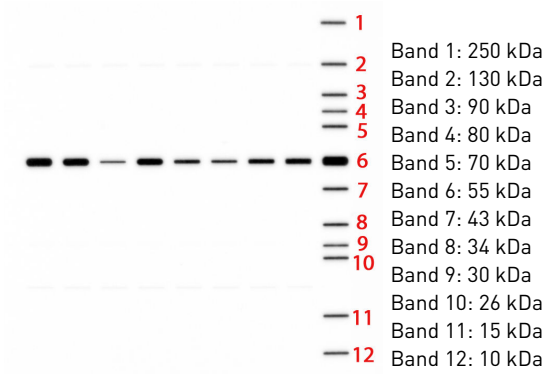
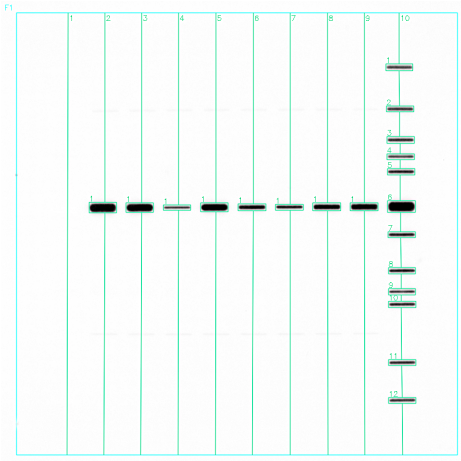


Fig. 7 Alexa Fluor™ 680

3. Normalization can be performed with iBright™ Analysis Software. Use Alexa Fluor™ 488 channel as the normalization lane and Alexa Fluor™ 680 as the experimental protein target.



Name	Vol. (Int.)	Local Bg. Corr. V...	Area	Rf	Density	Local Bg. Corr. D...	Rel. Quant.	Norm. Factor	Norm. Signal
LANE 1									
LANE 2									
BAND 1	31,778,763	28,315,255	1,020	0.44	31,155	27,760	4.253	1	28,315,255
LANE 3									
BAND 1	29,006,891	25,639,228	918	0.44	31,597	27,929	3.851	0.991	25,878,947
LANE 4									
BAND 1	7,901,197	6,657,271	561	0.44	14,084	11,866	1	0.99	6,727,677
LANE 5									
BAND 1	26,785,876	23,714,193	867	0.44	30,894	27,352	3.562	1.019	23,264,400
LANE 6									
BAND 1	15,711,432	13,805,084	728	0.439	21,581	18,963	2.074	1.004	13,755,244
LANE 7									
BAND 1	11,477,492	9,896,534	624	0.439	18,393	15,859	1.487	0.979	10,110,799
LANE 8									
BAND 1	19,574,773	17,343,899	760	0.439	25,095	22,235	2.605	0.911	19,034,181
LANE 9									
BAND 1	22,168,298	19,510,601	760	0.439	28,420	25,013	2.931	0.783	24,927,356

Fig. 8 Alexa Fluor™ 680

Limited product warranty

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