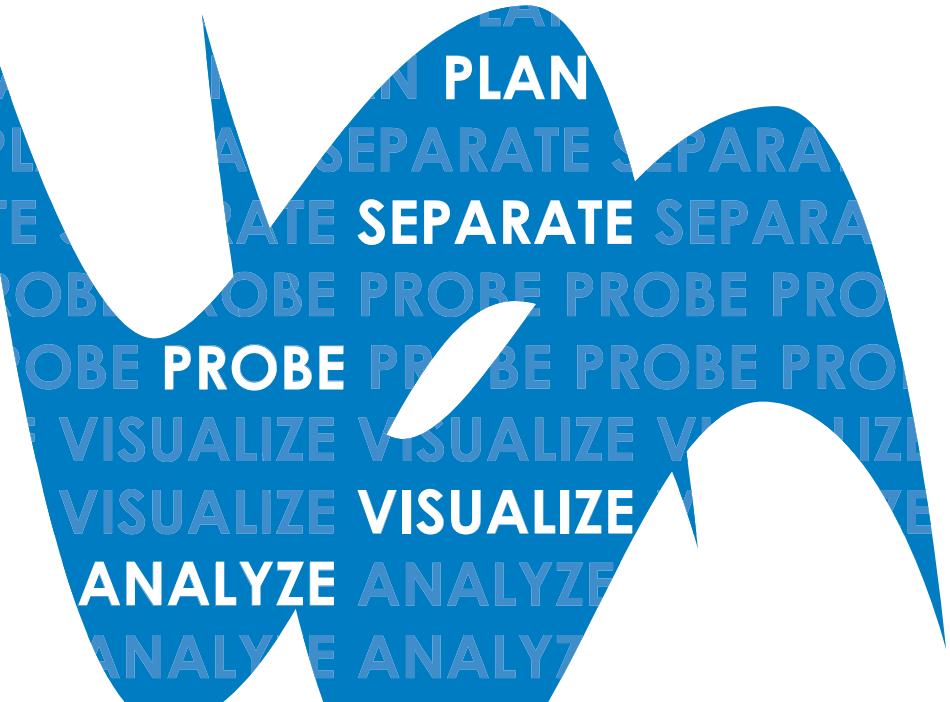
azure biosystems

TIPS

Transition from Chemiluminescent to Fluorescent Western Blots



Bring fluorescent Western blotting to your lab. It's easier than you think.

Chemiluminescence is the most familiar method of detection for Western blotting and offers great sensitivity. However, many scientific questions and experimental designs require the additional information provided by fluorescent Western blotting; this includes precise quantitation and visualization of similarly sized proteins within the same sample.

Once you make the decision to move to fluorescent Western blotting, what comes next? First, you can check if fluorescent Western blotting is right for your experiment using the flow chart in Figure 1. Then, refer to the tips and advice provided in this document to get started with your first fluorescent Western blot.

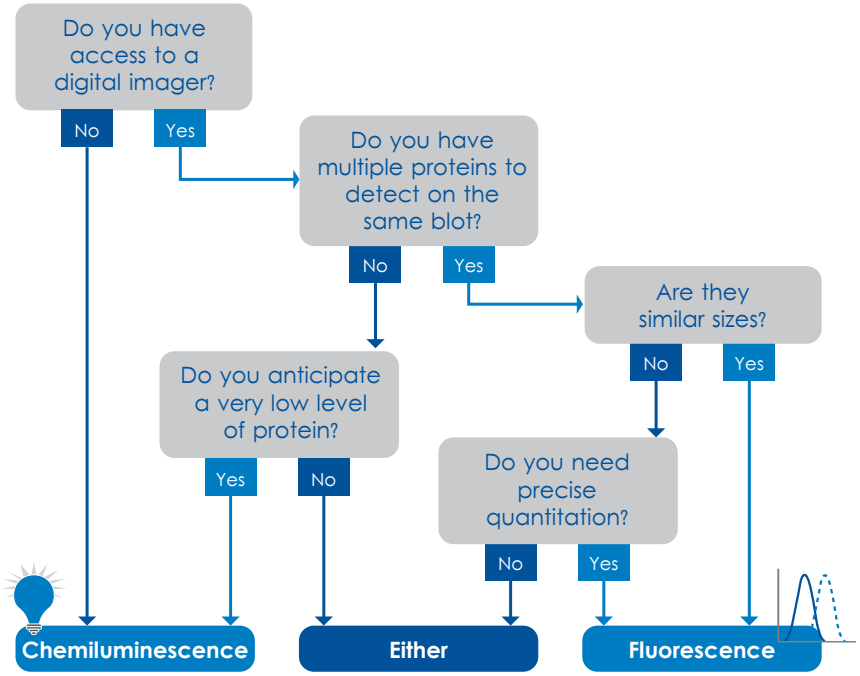


Figure 1. Chemiluminescent or Fluorescent Western Blotting.

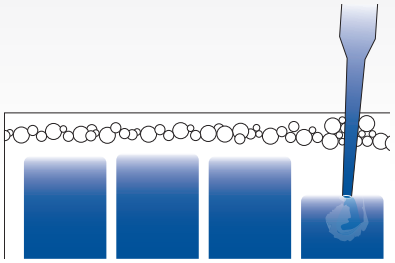
Before you run the gel

Dilute ladder

Some pre-stained protein ladders fluoresce strongly and can interfere with detection of proteins expressed at a low level. Try diluting the ladder 1 to 10 before loading.

Optimize sample concentration

As with any new assay, quality results are dependent on first optimizing your assay for the samples to be used. Sample load volume may need to be adjusted for the best possible results. When first switching from chemiluminescent to fluorescent Westerns, try a slightly higher sample load volume to confirm that your assay is working. Once you have a positive result, you can lower the sample load volume and push for more sensitive results.

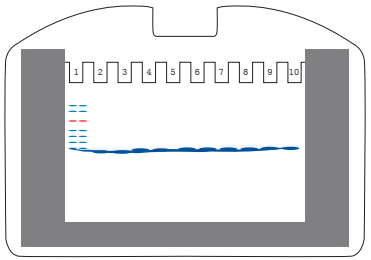


Make considerations for the protein ladder, sample concentrations, and loading dye.

While running the gel

Be wary of loading dye

Many loading dyes can fluoresce. To prevent this from interfering with the signal on a fluorescent blot, run the dye front off the gel or cut it off before transfer.



Before setting up the transfer, make sure to trim off the loading dye if possible to prevent it from interfering with your data.

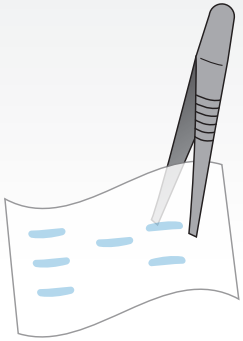
Transferring to the membrane

PVDF membranes

For the best signal-to-noise ratio, use a membrane with minimal autofluorescence such as Azure's low fluorescent PVDF membranes.

Membrane handling

Any contamination to the membrane will be obvious in a fluorescent Western blot. Always use forceps when handling membranes.



Use PVDF membranes and handle only with forceps.

Products

AC2108 – AC2109	Pre Cut PVDF

Probing the membrane

Blocking

As with chemiluminescent blots, it is important to completely block the membrane to avoid nonspecific binding of antibodies and to reduce background. Azure Protein Free Blocking Buffer and Azure Fluorescent Blot Blocking Buffer are formulated to not only reduce background, but also to stabilize the fluorescent dyes of AzureSpectra secondary antibodies for enhanced signal detection.

Optimize antibodies

Antibody concentration may need to be optimized and adjusted for the best possible results. When first switching from chemiluminescent to fluorescent Westerns, use the manufacturer's recommendations for antibody dilutions.

Incubation trays

Fluorescent secondaries can become quenched when exposed to bright light for long periods of time. When incubating your sample with light sensitive antibodies, cover your blot with an opaque material (such as with Azure's opaque incubation trays) to protect from quenching.

Products

AC2112	Azure Protein Free Blot Blocking Buffer
AC2190	Azure Fluorescent Blot Blocking Buffer
AC2128 – AC2139, AC2156 – AC2171	Fluorescent secondary antibodies
AC2120 – AC2123	Opaque Incubation Trays

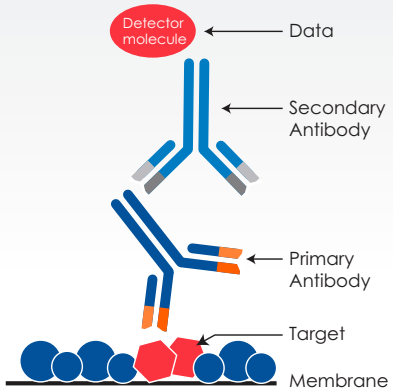


Diagram of the antigen-antibody and antibody-antibody interactions in Western blotting detection.

Use opaque containers to protect the light-sensitive fluorophores during incubation.

Washing

Stringent wash

Because the fluorescent dyes can adhere to the membrane, washing is extremely important to reduce background. Make sure to use a stringent wash, especially when working with near-infrared fluorophores. Azure Fluorescent Blot Wash Buffer is specially formulated for use with fluorescent Western blots.

Wash volume

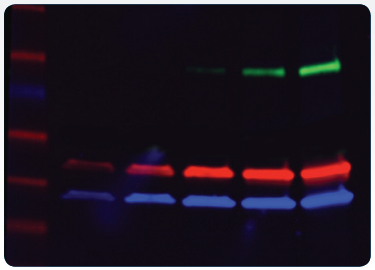
The volume and duration of washing is also important to rid the membrane of any free dye and antibody. We recommend two quick rinses in 25mL wash buffer followed by three 5 minute washes in 25mL wash buffer.

Final rinse

Any detergent in the wash buffer can also fluoresce, so rinsing in PBS or TBS after the final washing step is essential to lower background signal.

Products

AC2113	Azure Blot Washing Buffer
AC2145	Azure Fluorescent Blot Washing Buffer



To prevent background signals and get the clearest data, follow the recommended steps such as thorough washes after antibody incubation.

Imaging

Reduce background contamination

When many people use the same imager, the insides and trays can become contaminated. Background Quenching Sheets absorb background fluorescence to improve signal-to-noise ratios.

Products

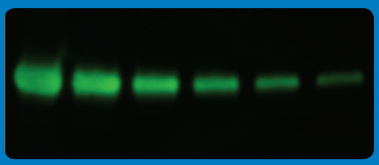
AC2144, AC2147	Quenching sheets

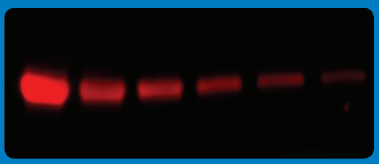
To prevent background fluorescence from interfering with your signal, use Azure's quenching sheets.

Want some help getting started?

A demo kit is a great way to try out fluorescent Western blotting in your own lab with your own samples and primary antibodies. It contains everything you need to perform single color fluorescent Western blotting:

- PVDF Membrane
- Fluorescent Blot Wash Buffer
- Fluorescent Blot Blocking Buffer
- Secondary Antibody
- 2 Quenching Sheets





AzureSpectra Demo Kits

AC2172	Goat α-Mouse 700
AC2173	Goat α-Rabbit 700
AC2174	Goat α-Mouse 800
AC2175	Goat α-Rabbit 800
AC2176	Goat α-Mouse 650
AC2177	Goat α-Rabbit 650
AC2178	Goat α-Mouse 550
AC2179	Goat α-Rabbit 550

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