Troubleshooting & FAQ

Western blotting can require substantial optimization due to the multiple steps involved. The correct amount of protein to load on the gel and the best dilutions of primary and secondary antibodies must be determined empirically. Some common questions are addressed below:

Problem	
What primary antibody dilution should we use?	Follow the manufacturer's recommendations for the antibody dilution. Typical antibody dilutions for primary antibodies range from 1:250 to 1:5000.
Can we use nitrocellulose membranes?	We recommend only "low fluorescence" PVDF membranes for high sensitivity fluorescent Western blotting applications.
What kinds of transfer methods are acceptable for use with the kit?	We suggest using a standard tank transfer method. Azure Transfer Buffer can be used for a quick wet transfer.
Can we use the blocking solution and/or wash solution we typically use?	We advise against using alternate blocking or washing solutions. However, if your primary antibodies have significant non-specific cross-reactivity with other proteins or with IgG, you can use the Azure Blocking Buffer provided with the kit as a base to prepare your specific antibody diluent solution.
We do not detect signal on the blot.	Check if the transfer was successful by using a protein standard; if this is positive, check the imaging system to confirm the correct excitation and emission settings. If you are trying to detect small amounts of a target protein, try to increase the concentration of your primary antibody first. If this is unsuccessful, also increase the concentration of the secondary conjugates.

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AzureSpectra rb650/ms550 Western Kit, with Protein Free Block

Quantitative, multi-color fluorescent Western blotting kit

Short Protocol for Catalog Number

AC2104 AzureSpectra rb650/ms550 Western Kit, with Protein Free Block

DC0005-002

azure biosystems

Storage Information

Unused AzureSpectra rb650/ms550 Western Kit AC2104 can be stored at +4°C before opening. After opening, some components of the kit may be stored at room temperature. Please see the labels on each component.

Warnings and Precautions

- For research use only. Not for clinical use. Not for internal use in animals or humans. Not for diagnostic use. Not for household or any other unintended use.
- Wear protective clothing such as protective glasses, gloves, and appropriate laboratory coveralls. Avoid contact with skin or eyes.
- Refer to appropriate MSDS or safety statement document for more information.
- All solutions included in the kit contain 1 µg/ml pentachlorophenol as a preservative against bacterial growth. Pentachlorophenol is a hazardous material. However, at 1 µg/ml it does not require any special handling beyond standard laboratory safety practices. When diluted to final working concentrations as directed in the Protocol, it no longer provides an anti-bacterial protection. Prepare only as much of each reagent as necessary to complete your current experiment.

Short Protocol

Important note: These volumes were determined based on the size of the tray needed to fit a 7 x 9 cm membrane and have the blot be fully covered.

1. Prepare your protein blot.
2. Prepare 30 ml of 1x Azure Protein Free Blot Blocking Buffer. Block membrane for 10 minutes in 10 ml of 1x Azure Fluorescent Blot Blocking Buffer.
3. Incubate blot for one hour at RT with primary antibody diluted in 10 ml 1x Azure Fluorescent Blot Blocking Buffer solution.
4. Prepare 300 ml of 1x Azure Fluorescent Blot Washing Solution. Wash blot with 1x Azure Fluorescent Blot Washing Solution:
   - 2x quickly
   - 3 x 5 min with 25 ml each.
5. Incubate blot with secondary antibodies diluted 1:2,500 (4 µL each) in 10 ml of 1x Azure Protein Free Blot Blocking Buffer. Note: mix gently by inversion when preparing secondary antibody solution.
6. Wash blot with 1x Azure Fluorescent Blot Washing Solution:
   - 2x quickly
   - 3 x 5 min with 25 ml each time
   - 1 x 5 min with 20-50 ml PBS or TBS without detergent.
7. Place blot on background quenching sheet and drain excess liquid; blot may be imaged immediately, or stronger signal may be obtained by waiting 15 to 30 minutes for membrane to become semi-dry.
8. Image using CCD camera; using the settings for imaging Cy3 and Cy5 will work well for the AzureSpectra conjugates.