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	Possible Solutions
What are your recommendations for primary antibody dilution?	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?	Nitrocellulose membranes have very high autofluorescence. We recommend only special "low fluorescence" PVDF membranes are suitable 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. Advansta's FLASHBlot Buffer can be used for a quick wet transfer.
Can we dry the blot and image [n] days after we do the Western assay?	The best performance for the blot is attained within 2 hours of drying it.
We have a blocking solution and/or wash solution we typically used; can we integrate those components into the kit methods?	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 AdvanBlock-PF blocking solution provided with the kit as a base to prepare your specific antibody diluent solution. If you intend to use your blocker, we suggest using AdvanBlock-PF for membrane blocking, and using your specific blocker to prepare the primary antibody incubation 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.
Our lab generally uses Tris/Phosphate based buffers; will this work with the kit?	We suggest using the Blocking and Washing solutions supplied with the kit to guarantee best performance. However if you require different buffer conditions, test a small blot before using larger quantities of kit components.

For detailed troubleshooting guide, please visit our website at www.advansta.com/Troubleshooting

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
Tel: 650.325.1980 | Fax: 650.325.1904
Email: support@advansta.com
www.advansta.com

WesternBright™ MCF

Quantitative, multi-color fluorescent Western blotting kit

For Catalog Number

K-12021-010 WesternBright™ MCF fluorescent Western blotting kit (Goat-anti-rabbit IgG APC/ Goat-anti-mouse IgG RPE)

advansta

August 17, 2018 | D-26026-408

Storage Information

Unused MCF kits K-12022-010 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. Do NOT freeze below 0°C.

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.

For More Information

including a detailed manual, visit **www.advansta.com/WesternBright_MCF** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

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.

Step

1. Prepare your protein blot
2. Prepare 20 ml of 1x AdvanBlock buffer. Block membrane for 10 minutes in 10 ml of 1x AdvanBlock.
3. Incubate blot for one hour at RT with primary antibody diluted in 10 ml 1x AdvanBlock blocking solution
4. Prepare 100 ml of 1x AdvanWash washing solution. Wash blot with 1x AdvanWash washing solution:
   - 1 x quickly with 10 ml
   - 1 x 15 min with 10 ml
   - 3 x 5 min with 10 ml each
5. Incubate blot with secondary antibodies diluted 1:2,500 (4 µL each) in 10 ml of 1x AdvanWash. **Note:** mix gently by inversion when preparing secondary antibody solution.
6. Wash blot with 1x AdvanWash washing solution:
   - 3 x 5 min with 10 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; for most instruments the settings for imaging Cy3 and Cy5 will work well for the WesternBright MCF conjugates