WesternBright™ MCF

Frequently Asked Questions

Questions	Answers
What primary antibodies can be used with WesternBright MCF?	WesternBright MCF is designed to work with primary antibodies raised in mouse and rabbit. Polyclonal or monoclonal antibodies can be used.
Must I use the AdvanBlock blocking agent?	The active component in the AdvanBlock solution not only provides quick and efficient blocking of the membrane from non-specific protein binding, but it also stabilizes the 3-D structure of the RPE and APC fluorophores and preserves their fluorescence when the stained blot dries out. If AdvanBlock is not used, the intensity of fluorescent signals will be greatly reduced after the blot has dried.
My primary antibody works best with a protein blocking agent – what can I do?	A protein blocking agent such as dried milk or BSA can be dissolved in AdvanBlock and used to dilute the primary antibody. Alternatively, you can use your optimal blocking reagent during the incubation with your primary antibody, and then use AdvanBlock to rinse the blot after the final wash to preserve the fluorescence of RPE and APC fluorophores. However, you must do one more final wash with a standard PBS or TBS buffer without any detergent to increase the stability of fluorescent signals and reduce general background.
Must I use the AdvanWash washing solution?	AdvanWash solution is made in a controlled environment using dedicated equipment and reagents tested and qualified for low autofluorescence. You may substitute AdvanWash with your own washing solution, but this may result in an increased fluorescent background.
Must I use the low fluor PVDF membranes from Advansta?	Autofluorescence of PVDF membranes from different vendors vary significantly. Our membranes were selected for their low autofluorescence. If you choose to substitute our PVDF membranes with your own, you will need to evaluate it for the level of autofluorescence. Some optimization of the protocol may be also required with different membranes.

What is the best transfer method to use with WesternBright MCF?

Most tank transfer procedures work well with WesternBright MCF. It is important not to exceed the recommended time and voltage values. Exceeding the voltage and/or duration of the transfer causes the accumulation of electrolysis by-products in the buffer and on the membrane, which results in increased general background, especially in the red excitation channel. We prefer the low-voltage tank transfer procedure described by M.W. Bolt and P.A. Mahoney in Analytical Biochemistry, 247, 185-192 (1997). Briefly, blotting is conducted for 2 hours at 10 V. Using this technique transfer can be also done at 24 V as quickly as 25 minutes. For such quick method, it is recommended that all part of the transfer apparatus are pre-cooled and the transfer performed in a cold room or in a refrigerator. Prepare the transfer buffer at least one day before using. Store the transfer buffer at room temperature, and do not pre-cool the transfer buffer. The transfer buffer used is 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 7.4, 20% (v/v) methanol, 0.05% (w/v) SDS.

Can I use a semi-dry transfer with WesternBright MCF?

We tested various semi-dry transfer methods and found most of them not satisfactory for fluorescent Western blotting applications using WesternBright MCF and other alternative fluorescent secondary antibodies. Semi-dry transfer methods cause high and non-uniform fluorescent background, especially in the green excitation channel.

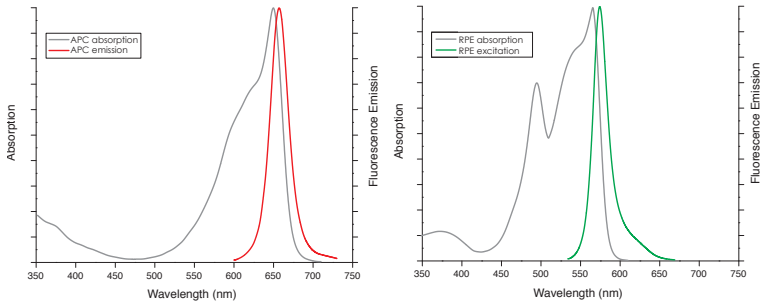
What imaging systems are compatible with WesternBright MCF?

Any imaging system capable of detecting Cy3 and Cy5 or similar dyes can be used to image blots detected with WesternBright MCF, including:

- FluorChem Q (Cell Biosciences)
- Typhoon 9400 (GE Healthcare)
- Ettan DIGE Imager (GE Healthcare)
- 4000 MM and 4000 MM Pro (Carestream Molecular Imaging)

What excitation and emission wavelengths are optimal for imaging blots detected with WesternBright MCF?

The excitation and emission spectra of the WesternBright conjugates are shown below. Specific settings recommended for specific imaging systems can be found in the WesternBright users manual.



I am experiencing high background – what can I do?

Some recommended steps to reduce background include:

1. Try a more-dilute solution of primary antibody.
2. Make sure you are using low-fluorescence PVDF membranes and background quenching sheets.
3. If you use your own washing solution, make sure that it is prepared from high quality reagents free from highly fluorescent impurities.
4. Add an additional final wash step, washing the membrane for 5 minutes with a standard PBS or TBS buffer that does NOT contain Tween-20 or any other detergent.
5. Try more dilute solution of secondary antibody
6. If polyethylene gloves are not available, use nitrile gloves and wash your hands in gloves with mild liquid soap and rinse thoroughly with deionized water. However, do not use antibacterial soap because some antibacterial agents may cause significant fluorescent background.

What concentration of primary antibody is recommended?

Optimal primary antibody concentrations must be determined empirically. A good starting point is the concentration suggested by the primary antibody provider, but Advansta recommends trying initial primary antibody concentrations in the range from 0.1 µg/ml to 0.5 µg/ml.

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