WesternBright™ ECL

Frequently Asked Questions

Questions	Answers
What secondary antibodies can be used with WesternBright ECL?	WesternBright ECL is designed to work with secondary antibodies conjugated to HRP, such as Advansta product numbers R-05072-500 and R-05071-500.
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 background. You may substitute AdvanWash with your own washing solution, but this may result in an increased background.
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 Background Quenching Sheets while imaging. 3. If you use your own washing solution, make sure that it is prepared from high quality reagents and using high purity water. Traces of metals in water may cause significant background. 4. Try increasing washing time. 5. Try a shorter exposure time. 6. Try a different blocking buffer. 7. Try more dilute solution of secondary antibody.
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.
What can I do if I am experiencing no or low signal?	1. Check that correct primary antibody used. 2. Check that secondary antibody recognizes primary (for example if the primary is a rabbit antibody, that the secondary is goat-anti-rabbit).
What can I do if I am experiencing white spots within bands?	Improve transfer, making sure to remove any bubbles between the gel and the membrane.
What can I do if I am experiencing speckled background?	1. Filter blocking and washing buffers. 2. Ensure that the laboratory environment is clean, to minimize dust, debris or any other particles that might come in contact with the blot. Cover the dish during incubation or washing steps. 3. Use non-powdered gloves, or switch to a different kind of glove. We recommend powder-free nitrile gloves or polyethylene gloves. 4. Filter secondary antibody.

Advansta Corporation
1455 Adams Drive, Ste. 1160 | Menlo Park, CA 94025
Tel: 650.325.1980 | Fax: 650.325.1904 | Email: sales@advansta.com
www.advansta.com