Fluorescent Western Blotting Using StarBright Dye Secondary Antibodies

Protocol

WB

This western blot protocol provides a procedure for using StarBright™ Blue Secondary Antibodies for fluorescent detection. The following immunodetection procedure has been found to produce clear, highly sensitive western blots. The StarBright Blue Secondary Antibodies can also be used successfully in many different immunodetection protocols.

Specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with the product and batch specific information provided with each antibody vial. A certain level of technical skill is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular cell types or applications.

Reagents

- Tris buffered saline (TBS)
- 1x TBS with 1% Casein (#1610782)
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- EveryBlot Blocking Buffer (#12010020)
- TBS + 0.05% Tween 20 (TBST)
- Fluorescence polyvinylidene fluoride (LF PVDF) membrane
- 0.02% sodium dodecyl sulfate (SDS)

Secondary Antibody Preparation

Resuspend the lyophilized content of the StarBright Blue Secondary Antibody tube in the indicated volume on the product datasheet of distilled or deionized water and leave on ice for at least 30 min prior to use. The resuspended solution may be stored at 4°C in the dark for up to 6 months, do not freeze.

Brief centrifugation (pulse spin for 2–3 sec at max speed in a tabletop microcentrifuge) can be employed to collect the contents to the bottom of the tube. Caution: do not use prolonged centrifugation (more than 10 sec).

Method

This method begins after protein separation and membrane transfer.

All steps are at room temperature (RT) (22–25°C) except for the overnight primary incubation.

1. Block: Incubate for 5 mins in Blocking Buffer (12010020), block for 5 min.
2. Incubate in primary antibody: Dilute and incubate the primary antibody as specified in your protocol or by the supplier. If no protocol is provided with the primary antibody, it can be diluted in TBS + 1% casein buffer or EveryBlot Blocking Buffer.
3. Wash: 5 x 5 min at RT with TBST (TBS + 0.05% Tween 20).
4. Incubate with StarBright Blue Secondary Antibody: Dilute secondary antibody 1:2,500 in TBS + 1% casein or EveryBlot Blocking Buffer. If using low fluorescence polyvinylidene fluoride (LF PVDF) membrane, also add 0.02% sodium dodecyl sulfate (SDS). For nitrocellulose, do not add SDS. Note: the presence of 0.02% SDS in the StarBright incubation buffer for LF PVDF blots does not affect the performance of other fluorescent antibodies, such as DyLight 800 antibodies or hFAB Rhodamine Anti-Housekeeping Protein Primary Antibodies.
5. Wash: 6 x 5 min at RT with TBST.
6. Image using a fluorescent imager. The ChemiDoc™ MP Imaging System or ChemiDoc Go Imaging System are ideal as the Image Lab Touch Software has a specific setting for these two StarBright Blue Dyes. Select StarBright B520 or StarBright B700 under Application > Blots.

Notes:

- SDS-PAGE gels are run in the conventional manner and transblotted onto LF PVDF or nitrocellulose using equipment and methods of choice. Precision Plus Protein All Blue Prestained Protein Standards are recommended (suggested loading volume: 5 µl). We do not recommend standards that include pink or multicolor bands since these can exhibit fluorescence that may interfere with imaging
- StarBright Blue Secondary Antibodies may be used for detection on LF PVDF or nitrocellulose membranes
- For blocking and washing, use 15 ml for a mini gel. For primary and secondary antibody incubations, use 10 ml for a mini gel. Use a flat-bottom tray that is as small as possible while still accommodating the blot
- Place aluminium foil over the incubation tray during all incubations after the fluorescent antibody is added to avoid photobleaching. Image immediately after the final wash for best results
- Do not allow the blot to dry out at any time during incubations and washes
- Shake or rock well (without spilling) during incubations with StarBright Blue Secondary Antibodies
- Dilute StarBright Dye–conjugated antibodies into the incubation solution immediately prior to use
- For multiplex experiments, we recommend detecting the target proteins with the least abundance using the StarBright Blue 700 Secondary Antibody because the label has enhanced brightness compared to other traditional dyes
- The presence of detergents can contribute to fluorescence background and therefore should be used with caution. Do not add Tween 20 to solutions containing StarBright Dye–conjugated antibodies
- Membranes should be handled only by their edges and only with clean forceps. Take great care to never touch the membrane with bare or gloved hands
- In general, keep everything clean. Prevent background by thoroughly cleansing all equipment and trays prior to use. If reusing staining trays, clean with laboratory detergent or dish soap prior to use
- At all blocking and antibody incubation steps, ensure that the entire surface of the membrane is covered and keep the trays covered during all incubations
- Appropriate controls should always be carried out. It may be useful to include a sample in which no primary antibody is used at all, in order to determine any nonspecific binding of the secondary reagent to the target tissue

Visit bio-rad-antibodies.com/western-blotting for more information on western blotting.

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