Storage Information

The OneBlock™ Western-FL reagent is stable at 4°C for at least one year.

Warnings and Precautions

- OneBlock™ Western-FL is for research use only.
- Always wear gloves when handling reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the enclosed protocol is followed by properly trained personnel.

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Protein Biology Products

Short Protocol

OneBlock™ Western-FL Blocking Buffer

Antibody-antigen signal enhancing and blocking solution for fluorescent Western Blots

For Catalog Numbers

Cat # 20-314 OneBlock™ Western-FL, 1L

Description

OneBlock™ Western-FL is a novel blocking solution, optimized to enhance specific antibody-antigen interactions for fluorescent Western Blots. This all-in-one blocking and antibody incubation solution is designed to improve sensitivity and decrease overall background. Non-specific binding caused by low quality antibodies is reduced while signal from the specific antibody-antigen complex is stabilized and enhanced. Provided as a convenient ready-to-use solution intended to directly replace other commonly used blocking buffers for Western Blotting.

OneBlock™ Western-FL

Short Protocol

Steps

1. Prepare your protein blot on either a PVDF or Nitrocellulose membrane using your standard technique.

2. Block the membrane for one hour at ambient temperature with gentle agitation using a sufficient volume of buffer to completely cover the membrane.

3. Incubate with the primary antibody diluted in OneBlock™ Western-FL for one hour at ambient temperature with gentle agitation.

4. Wash the blot with washing solution, PBST or TBST:
   - 2 x quickly (~5 seconds per rinse)
   - 3 x 5 minutes, with at least 0.3 mL/cm^2 membrane each time

5. Incubate with the secondary antibody diluted in OneBlock™ Western-FL for one hour at ambient temperature with gentle agitation.

6. Wash the blot with washing solution, PBST or TBST:
   - 2 x quickly (~5 seconds per rinse)
   - 3 x 5 minutes, with at least 0.3 mL/cm^2 membrane each time

7. Rinse the blot for 5 minutes with 1X PBS to remove detergent which may cause elevated fluorescent background.

8. For best results, image the blot dry.

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Tips

- Western blotting requires optimization of primary and secondary antibody concentrations used in steps 3 and 5 of the Short Protocol. These must be determined empirically for every antigen-antibody pair.
- Fluorescent Western blotting can be performed as a common procedure utilizing a primary antibody and a fluorescently labeled secondary antibody. Alternatively, directly labeled primary antibodies may be used, which eliminates the need of secondary antibody and shortens the procedure. Adjust the protocol appropriately if using directly labeled primary antibodies.
- OneBlock™ Western-FL increases sensitivity, so optimal antibody concentrations may be lower than with other blocking buffers.
- For IR dyes use washing solution for decreased non-specific background.
- Make sure not to touch the membrane with fingers or dirty forceps as this can result in non-specific background.