Tips

- Western blotting requires optimization of primary and secondary antibody concentrations. These must be determined empirically for every antigen-antibody pair.
- OneBlock™ Western-CL increases sensitivity, so optimal antibody concentrations may be lower than with other blocking buffers.
- For film detection, use antibody concentrations 2-5 fold lower than for CCD imaging.
- Make sure not to touch the membrane with fingers or dirty forceps as this can result in non-specific background.

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PROMETHEUS™
Protein Biology Products

Short Protocol

OneBlock™ Western-CL Blocking Buffer

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

For Catalog Numbers

Cat # 20-313	OneBlock™ Western-CL, 1L

OneBlock™ Western-CL Blocking Buffer

Description

OneBlock™ Western-CL is a novel blocking solution, optimized to enhance specific antibody-antigen interactions for chemiluminescent 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.

Storage Information

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

Warnings and Precautions

- OneBlock™ Western-CL 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.

Short Protocol

Steps

1. Prepare your protein blot on either PVDF or nitrocellulose 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 the blot with the primary antibody diluted in blocking solution 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 the blot with the secondary antibody diluted in blocking solution 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. During the final washing step, prepare a working solution of chemiluminescent substrate.

8. Thoroughly drain all wash solution from the blot then apply the working solution of the chemiluminescent reagent to the blot (use 0.1 ml/cm^2 of your membrane). Incubate the blot with the reagent for 5 minutes.

9. Drain excess substrate and place the blot in your CCD imager and image. If a long exposure is required or if imaging will be performed using X-ray film, place the blot in a blot development folder for best results.