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.
- Azure Protein-free Blot Blocking Buffer increases sensitivity, so optimal antibody concentrations may be lower than with other blocking buffers.
- For IR dyes use Azure Fluorescent Blot Washing Buffer 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.

Note

- Azure Biosystems provides a reliable technology to prepare fluorescently labeled antibodies in an easy to use AzureSpectra Fluorescent Antibody Labeling Kits format.

www.azurebiosystems.com • info@azurebiosystems.com

Protein-free Blot Blocking Buffer

Non-protein blocking solution for fluorescent and chemiluminescent Western blots

Short Protocol for Catalog Number

AC2112 Azure Protein-free Blot Blocking Buffer, 200mL

Description

Azure Protein-free Blot Blocking Buffer is a protein-free blocking solution, optimized for use with the AzureSpectra conjugates and also an excellent choice for chemiluminescent Western blot experiments. This fast-acting blocking solution stabilizes the fluorescence of the AzureSpectra conjugates. Azure Protein-free Blot Blocking Buffer can reduce background when used with primary antibodies that have a high degree of cross-reactivity with protein blockers such as BSA, casein,

SD-0173 R1

azure biosystems

or milk protein. With low-quality primary antibodies that may require protein-based blocking agents, BSA or non-fat dry milk can be dissolved directly in the Azure Protein-free Blot Blocking Buffer solution used to dilute the antibody. Provided as a 5X concentrate.

Storage Information

Azure Protein-free Blot Blocking Buffer reagent is stable at 4°C for at least one year.

Warnings and Precautions

- Protein-free Blot Blocking Buffer 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

1. Prepare your protein blot on either a PVDF or Nitrocellulose membrane using your standard technique.
2. Block the membrane for 10 minutes at ambient temperature with gentle agitation using a sufficient volume of Azure Protein-free Blot Blocking Buffer to completely cover the membrane.
3. Incubate with the primary antibody diluted in Azure Protein-free Blot Blocking Buffer for one hour at ambient temperature with gentle agitation.
4. Wash the blot with Azure Blot Washing Buffer or Azure Fluorescent Blot Washing Buffer, 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 Azure Blot Blocking Buffer for one hour at ambient temperature with gentle agitation.
6. Wash the blot with Azure Blot Washing Buffer or Azure Fluorescent Blot Washing Buffer, 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.