NH DyeAGNOSTICS

Product Guide

Immuno Blue Western Blotting Substrate

Product No. PR840

NH DyeAGNOSTICS GmbH
Weinbergweg 23
D-06120 Halle

Technical support
Phone: +49 (0) 345-2799 6413
e-mail: service@dyeagnostics.com
www.dyeagnostics.com

Revision 10/2014

Kit content (100 ml)

- Immuno Blue reagent A (100 ml)
- Immuno Blue reagent B (2.5 ml)

Sufficient for 40 minigel blots (4000 cm^2 of blotting membrane).

Storage

Store at 2°C to 8°C and avoid exposure to intense light.

Best before: see packaging

Additional materials required

- low fluorescent blotting membrane
- primary antibody
- secondary antibody conjugated with HRP
- blocking reagent
- dilution and wash buffer
- imaging device for blue fluorescence detection

General information

Immuno Blue is a highly sensitive fluorescent substrate for the dection of HRP-conjugated secondary antibodies. Immuno Blue raw substrate is converted to the fluorescent Immuno Blue reaction product, which remains on the blot. The detection is performed by fluorescence imaging. Depending on the performance of the imaging system and the abundancy of the target protein the exposure time is in the range of serveral seconds to one minute. The signal remains stable for at least 4 weeks.

Western blotting procedure

Note: The following instructions are generalized. Appropriate blocking reagent, wash buffer, antibody dilutions and incubation duration have to be determined experimentally for each application. Do not use sodium azide as a preservative. Sodium azide is an inhibitor of HRP.

1. Remove blotting membrane from the transfer unit and block non-specific sites with blocking reagent for 60 minutes at room temperature (RT) with shaking.

2. Remove the blocking reagent and add the primary antibody working dilution. Incubate blot for 1 hour at RT with shaking or overnight at 2°C to 8°C without shaking.

3. Remove primary antibody working dilution and briefly rinse blotting membrane in wash buffer.

4. Wash membrane by suspending it in wash buffer and agitating for 5 - 10 minutes. Replace wash buffer at least 3 times. Increasing the wash buffer volume, the number of washes and wash duration may reduce background signal.

5. Incubate blot with the secondary antibody (HRP-conjugated) working dilution for 1 hour at RT with shaking.

6. Repeat washing steps 3 and 4 to remove excess of secondary antibody. The membrane must be thoroughly washed after incubation with the HRP-conjugate.

7. Prepare the Immuno Blue working solution by mixing Immuno Blue reagent A and reagent B in a 40:1 ratio. Use at least 30 µl working solution per cm^2 of membrane.

Note: The Immun Blue working solution is stable for up to 45 min at room temperature.

8. Incubate blotting membrane with freshly prepared Immuno Blue working solution for 5 min at RT.

9. Remove blotting membrane from Immuno Blue working solution and briefly rinse with washig buffer two times.

10. Perform fluorescence detection using a fluorescence detection system with appropriate excitation and emission filter settings:

Absorption max.	485 nm
Emission max.	510 nm

The Immuno Blue fluorescence signal remains stable for at least 4 weeks. Store blotting membrane in the dark at RT.