NH DyeAGNOSTICS

# Immuno Blue Western Blotting Substrate

Product no. PR840

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FOR RESEARCH USE ONLY

## 1 Products and content

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

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

## 2 Storage and stability

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

Best before: see packaging

## 3 Safety instructions

May cause respiratory irritation. Extremely flammable liquid and vapour. May be harmful if swallowed. Causes serious eye irritation. Suspected of causing cancer.

## 4 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

## 5 General Information

Immuno Blue is a highly sensitive fluorescent substrate for the detection 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.

## 6 Instructions for Use

*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.*

- Remove blotting membrane from the transfer unit and block nonspecific sites with blocking reagent for 60 minutes at room temperature (RT) with shaking.
- 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.
- Remove primary antibody working dilution and briefly rinse blotting membrane in wash buffer.
- Wash membrane with wash buffer for 5 - 10 minutes. Repeat at least 3 times. Increasing the wash buffer volume, the number of washes and wash duration may reduce background signal.
- Incubate blot with the secondary antibody (HRP-conjugated) working dilution for 1 hour at RT with shaking.
- Remove secondary antibody working dilution and briefly rinse blotting membrane in wash buffer.

- Wash membrane with wash buffer for 5 - 10 minutes. Repeat at least 3 times. Increasing the wash buffer volume, the number of washes and wash duration may reduce background signal.

- 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 Immuno Blue working solution is stable for up to 45 min at room temperature.*

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

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

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

  Absorption max. 435 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.