

# UP40301 SeaBlock™ saturating agent

## Product Description

<b>Name:</b>	SeaBlock™ IA saturating agent, for ImmunoAssays
<b>Part Number :</b>	UP40301A, 500ml
<b>Storage:</b>	+4°C
<b>Self life :</b>	1 year from date of receipt

Fish serum based saturating agent formulated for the immuno-enzymatic detection techniques, especially ELISA and Blotting. This reagent suits chromogenic and chemiluminescent systems, as well as immunochemistry, improving results in most applications where antibodies generate a background with other standard reagents.

## Direction for Use

Note: These are standard protocols for western blotting and ELISA.

### Protocol for western blotting

The protein(s) of interest should be separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE). After separation the proteins need to be transferred to nitrocellulose or other suitable membrane (PVDF, Nylon...).

Follow the protocol recommended by the manufacturer of the electrophoresis and blotter apparatus.

- Block the membrane for at least one hour shaking in an adequate volume of SeaBlock™ 1X at room temperature.

Wash once with PBS, Tween 0.05%. The blot can be stored at +4°C.

Note: 25 ml is generally the right volume to incubate minigels 8x8 cm<sup>2</sup>.

Seablock concentration may be optimized in the 10-100% range, in your normal blocking buffer completely substituting your existing protein (BSA,CAH,etc.).

- Incubate with the primary antibody under agitation during 1 h in PBS Tween 0.05% with 2% of SeaBlock™ agent.

Antibody concentration should be determined (usually 0.1-10µg/ml for monoclonals), depending on their affinity, and some antibodies may require up to 2h incubation.

Seablock concentration may be optimized in the 1-10% range, in your normal blocking buffer completely substituting your existing protein (BSA,CAH,etc.).

- Wash the membrane extensively (usually 30 minutes with at least 3 changes of buffer, with PBS, Tween 0.05%).

- Incubate with the secondary antibody in PBS, Tween 0.05% with 2% of SeaBlock™ agent, 1h at room temperature.

This was used successfully for rabbit, mouse and/or rat primary antibodies / corresponding secondary antibody.

**Ask Uptima** for the right antibody. For example, optimal results were obtained with Goat anti mouse-HRP #UP446330

The appropriate serum, from the species of the primary antibody, can be included too.

- Wash extensively, at least 4 times with PBS Tween 0.05%.

For a chemiluminescent detection, up to 6 washes, and >1h total washes may be required to lower the background significantly without loss of sensitivity

- Incubate with the substrate according to the manufacturer protocol.

**Ask Uptima** for the right substrate.

For example, optimal results were obtained with chromogenic TMB ready solution #E865 and chemiluminescent UptiLight #UP99619A

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FT-UP40301

Nitrocellulose/Colloidal Gold Rapid Test Assay Formats

1.5 to 5% SeaBlock™ in your normal blocking buffer either completely substituting your existing protein (BSA,CAH,etc.) or varying combinations(SeaBlock™ 1.5% with BSA, etc.)

### Protocol for ELISA

- Coat the antigen in carbonate 0.1M buffer pH9.6, 100µl per well, overnight at +4°C  
PBS can also be used for alkaline labile antigens, and 2h room temperature incubation may be preferred, but with possible lower efficiency.
- Add 250µl of SeaBlock™ solution in ELISA wells, incubate 1h at +37°C  
Incubation can be optimized, in the 30 mn-4 h duration range, and done at room temperature.  
SeaBlock™ can be used pure, or diluted 1/2-1/20 for applications where the antigen seems difficult to detect.
- Wash once with PBS Tween 0.05%.
- Primary antibody at appropriate dilution in PBS Tween 0.05% + 2% SeaBlock™ agent, 1h at +37°C  
Antibody concentration should be determined (usually 0.1-10µg/ml for monoclonals), depending on their affinity, and some antibodies may require up to 2h incubation.

Seablock concentration may be optimized in the 0.75-10% range, in your normal blocking buffer completely substituting your existing protein (BSA,CAH,etc.) or varying combinations (SeaBlock™ 0.75% with BSA, etc.).

- HRP or PAL labeled secondary antibody at appropriate dilution PBS Tween 0.05%+ 10% SeaBlock™ agent, 1h at +37°C  
Ask Uptima for the right antibody. For example, optimal results were obtained with Goat anti mouse-HRP #UP446330
- Wash extensively, at least 4 times with PBS Tween 0.05%.
- Incubate the substrate according to the manufacturer protocol.  
Ask Uptima for the right substrate.

For example, optimal results were obtained with chromogenic TMB ready solution #664780 and chemiluminescent UptiLight #UP99620A

### Stabilizing Diluent For Antigen Preparations

1 to 2 % in your diluent. SeaBlock™ may be heated to inhibit enzyme activity. However, SEA BLOCK™ will gel if heated alone. SeaBlock™ will not gel if salts are added prior to heat treatment.

## Scientific and Technical Information

- SeaBlock™ IA is a fish-based formulation, supplied as a liquid conditioned in PBS (150 mM NaCl, Phosphate 20mM, pH 7.5) with 0.1% sodium azide.
- It is optimized for diagnostic systems involving membrane or other solid phase supports, notably ELISA and western Blotting. It also suits immunochemistry although. This reagent imparts superior stability and flow characteristics, while using proteins that do not interact with mammalian antibodies. This allows very low background to overcome saturating agents like milk, BSA, Gelatin... , especially for high binding surfaces, Glutaraldehyde activated Amine polystyrene.
- Standard features: SeaBlock™ like mammalian serum based saturants, contains a wide variety of biomolecules and thus prevents non-specific binding in the same effective manner. Also, it usually does not mask bound proteins. SeaBlock™ prevents the denaturation of proteins during immobilization or drying.
- Specific features: SeaBlock™ non-mammalian nature prevents interactions with immunoreagents (i.e. mammalian antibodies) that are observed for mammalian based saturants, and responsible of high background in some assays. The natural body temperature of fishes is room temperature and below. Therefore, SeaBlock™ is an excellent stabilizer for proteins when used as a diluent of reagents stored refrigerated or frozen. SeaBlock™ is obtained from domesticated fish stocks held under controlled conditions (genetics, environment, diet, and reproductive status) to ensure reproducibility and lot-to-lot consistency.
- Although SeaBlock™ / EIA / WB is designed to be used at 1x, we suggest experimenting with dilutions in PBS from 1:2 to 1:20, and it is sometimes enough effective at a concentration of 1% or less.

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Concentrations optimization is especially important in super sensitive western blot works. Best results with EIA/ELISA applications tend to be at lower dilutions and undiluted.

- SeaBlock™ agent works well with all detection systems : it notably suits biotinylated antibodies / (strept)avidin systems, in opposition to milk based saturating agents.
- One should keep in mind there is no ideal saturating agent for all antigen/application. In case of low detection of an antigen (masking), try a higher dilution of Seablock, or choose an other agent (ask Uptima for BSA agent #UP900100, or other agents). In case of undesired background, try a lower dilution of Seablock, or an other agent.

## Other Information

This product is sold for research purposes only. It is not to be used for humans or animals. There is no express or implied warranty. No liability is assumed merchantability, or direct and consequential damage. The user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with all regulations.

For any question, please **ask Uptima** or your local distributor.

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