



SeaBlock saturating agent

Product Description

SeaBlock is a unique, effective and safe reagent for blocking EIA applications.

Name: SeaBlock™ IA saturating agent - for ELISA and other ImmunoAssays

Catalog Number : UP40301A, 500ml

Storage: +4°C (K)
Stable > 1 year from date of receipt

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

SeaBlock™ products are derived entirely from fish, so:

- they **avoid cross-species reactivities observed with mammalian based agents**, such as BSA, fat milk, casein-based agents,...
- they are **not subject to the European Union ban** on all cattle products that could carry Bovine Spongiform Encephalopathy (**no BSE**).

Also available in different formulations: see [related products](#).

Directions for use

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

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°

Incubation can be optimized, in the 30 mn-4 h duration range, and done at room temperature.

SeaBlock™ can be diluted 1/2-1/20 when it is suspected to decrease the signal or if antigen remains difficult to detect. It can even be combined with other saturating agents. See [related products](#).

FT-40301A

- Wash once with PBS Tween 0.05%.
- Incubate the 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 monoclonal abs), depending on their affinity, and some antibodies may require up to 2h incubation.

Other buffers can be used as well (i.e. TBS). Seablock concentration may be optimized in the 0.75-10% range, completely substituting your usual saturating agent or in combination (i.e. SeaBlock™ 0.75% with BSA).

Incubate the HRP or PAL labeled secondary antibody at appropriate dilution PBS Tween 0.05%+ 10% SeaBlock™ agent, 1h at +37°C

It is recommended to use Uptima secondary antibodies (i.e. Goat anti mouse-HRP #UP446330)

- Wash extensively, at least 4 times with PBS Tween 0.05%.
- Incubate the substrate according to the manufacturer protocol.

Recommended substrates are i.e. TMB solution #664780 and chemiluminescent UptiLight #UP99620A. See [related products](#)

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 cm².

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 monoclonal abs), 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 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 may achieve lower background without loss of sensitivity
- Incubate with the substrate according to the manufacturer protocol.
Recommended substrates are i.e. TMB solution #CL2731 and chemiluminescent UptiLight #UP99619A&98490A. See [related products](#)

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

Nitrocellulose/Colloidal Gold Rapid Test Assay Formats

Add 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.)

Stabilizing Diluent For immunologicals or Antigen Preparations

Add 1 to 2 % to your diluent. SeaBlock™ may be heated to inhibit enzyme activity. However, SeaBlock 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.
- **Applications:** It is a effective agent for detection systems involving membrane or other solid phase supports, notably ELISA and western Blotting. It also suits immunochemistry. This reagent overcomes –notable through lower background- most of time saturating agents like non-fat milk, BSA, Gelatin, FBS... thanks to unique features (see below). It is excellent to saturate high binding surfaces, and Glutaraldehyde activated Amine polystyrene (when BSA is respectively a good but not excellent and a poor blocker). For blotting application, one may try an optimized formulation, AquaBlock #AM728.
- SeaBlock agent works well with all detection systems : it notably suits biotinylated antibodies / (strept)avidin systems, in opposition to milk based saturating agents.
- **Standard and specific features:** SeaBlock presents not only interesting features of mammalian saturants, but has also unique features:

Some of the Advantages of Seablock:

- **Reproducibility:** SeaBlock™ is obtained from domesticated fish stocks held under controlled conditions. This control of genetics, environment, diet, and reproductive status permits reproducibility and lot-to-lot consistency that would not be possible in sera from wild fishes.
- **Ethics:** Each fish is anesthetized, a few milliliters of blood drawn aseptically, and the fish is returned to the water- all under veterinary supervision.
- **Low Cross Reactivity:** SeaBlock prevents interactions with immunoreagents that are observed for mammalian-based saturants, and responsible of high background in some assays: the wide phylogenetic separation of fish in SeaBlock™ and mammals reduce risks of cross-reactivity with anti-mammalian IgG antibodies and anti-DNA or anti-BSA hapten antibodies. Since there is no IgG in SeaBlock , there is no cross-reactivity with Protein A.
- **Molecular Diversity:** SeaBlock like mammalian serum, contains a wide variety of biomolecules and thus prevents non-specific binding in the same/ effective manner. Like mammalian serum, SeaBlock™ does not mask bound proteins.
- **Stabilizes:** Bound Molecules SeaBlock prevents the denaturation of/ proteins during immobilization or drying. 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.
- **Effective:** SeaBlock is effective for most applications at a concentration of 1% or less.
- **Concentration:** Although SeaBlock IA 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. Concentrations optimization is especially important in super sensitive assays where best results may be at lower dilutions than undiluted. Alternatively try the AquaBlock #UPAM728.
- One should keep in mind there is no ideal saturating agent for all antigen/application. In case of undesired background, or of low detection of an antigen (masking), try a higher dilution of Seablock, or choose an other agent (see related products). Mixture of agents can even be tested.

Other information

Legals: for R&D use only

SeaBlock 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.

Related products: [search on line](#)

SeaBlock, serum free (for lateral flow systems) in PBS #UPAP1380 and in TBS #UPAP1370

AquaBlock #UPAM728 (optimized for supersensitive assay systems, excels in Western Blots)

Seagrow #DO7650 (derived from salmonids. Proven to work very well for zebrafish cell culture as a media additive)

BSA Biotech, 30% solution #UP900100

Non-fat Milk #768701

Gelatin N13360 - Bloom number: 240-270 ; pH(28°C): 4.5-5.5 ; Water: <12% ; Viscosity: 35-45mpa]

TBS with Non-Fat Powdered Milk 3% #GS4160; with BSA 1% #GS4170, with Tween® 0.05% #GS4200

PBS with Non-Fat Powdered Milk 3% #GS4180, with BSA 1% #GS4190, with Tween® 0.05% #GS4250

Antibody Diluent (Ready to Use) #HH6690

BioBlock membrane blocking agent #N13660 (in PBS), #N13650 (in TBS)

An economic standard blocker based on casein, optimized for nylon or PVDF membranes / blotting applications.

Tween® 20, pure #15874A, oxidant free 20% solution UP158740

Great HRP substrates ([TMB solutions](#); [UptiLight ECL reagents](#))

Uptima secondary antibodies (i.e. Goat anti mouse-HRP #UP446330)

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

rev. I03E-B08E