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# BlockAid™ Blocking Solution

## Catalog no. B10710

**Table 1** Contents and storage

| Material | Amount | Concentration | Storage* | Stability |
|---|---|---|---|---|
| BlockAid™ Blocking Solution | 50 mL | 1X | ≤–20°C | When stored as directed, the product is stable for at least 6 months. |

* For short-term storage (less than two weeks), BlockAid™ blocking solution can be stored at 2–8°C. Aliquoting is recommended.

## Introduction

Reducing non-specific binding of antibodies and other protein-dye conjugates is important for achieving the best possible signal-to-background in any immunofluorescent assay. This is particularly important when trying to detect low-expressing antigens or for super-resolution imaging. Though some background can be due to dye-charge binding, which can be blocked by the Image-iT® FX Signal Enhancer Solution (Cat. no. I36933), the primary cause of non-specific labeling is from non-specific protein binding.

Traditional protein blockers, such as casein or fish-skin gelatin, are often ineffective. Use of bovine serum albumin requires a highly-purified grade of product and relatively high concentration. Use of normal serum requires heat-inactivation to reduce proteases, and care should be given to match the species with the host species of the secondary antibody. Often, a combination of these components is necessary, requiring optimization and driving up costs per assay.

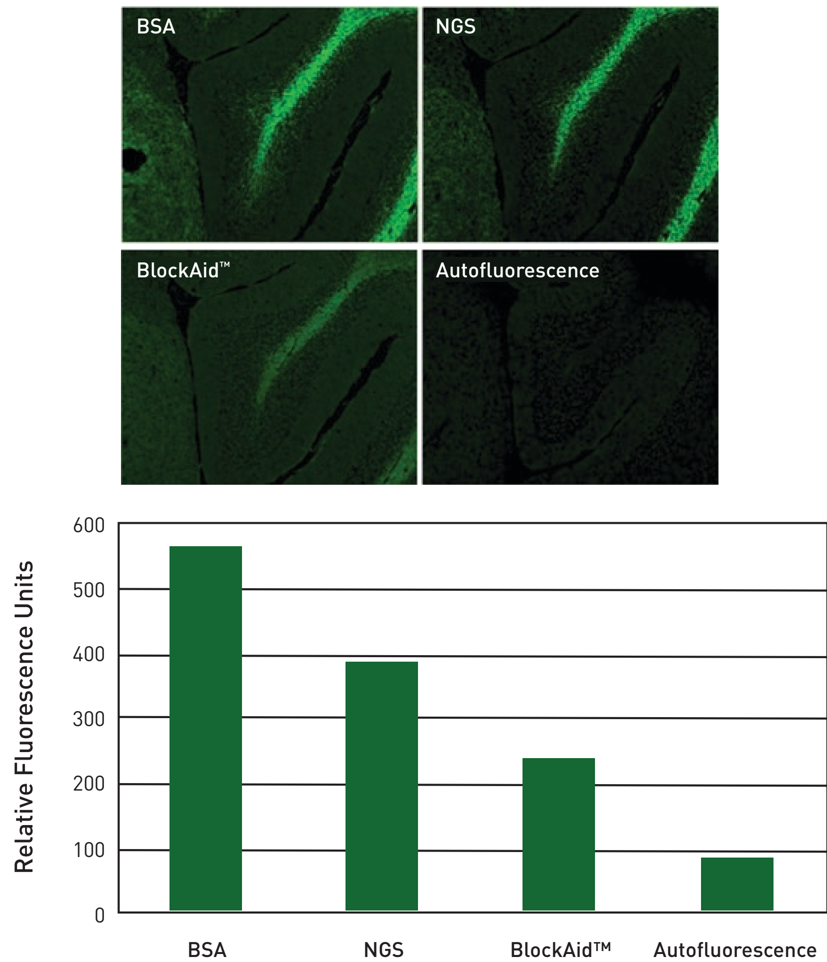
BlockAid™ blocking solution offers an optimized mix of protein blockers that has been demonstrated to be better at blocking non-specific protein binding than traditional compounds for immunofluorescent labeling of cultured cells, embryo whole mounts, and tissue sections (Figure 1, page 2). The solution is ready-made, without the need for further dilution or concern for the species of antibody. It works as an initial blocking solution, and it can be used for diluting both primary and secondary antibodies. BlockAid™ solution has also been shown to give superior blocking for streptavidin conjugates, Qdot® antibody conjugates, and for protein-microsphere conjugates.

For Research Use Only. Not for use in diagnostic procedures.

## Materials

BlockAid™ Blocking Solution comprises a mixture of proteins dissolved in phosphate-buffered saline (PBS) and contains 0.02% thimerosal as a preservative. The solution is supplied in a 50 mL unit size, which is sufficient for approximately 250 assays at 200 µL per assay. For long-term storage, BlockAid™ Blocking Solution should be stored at –20°C in convenient-sized aliquots. For short-term storage (less than two weeks), the solution can be stored at 2–8°C.

**Figure 1** Effectiveness of various blocking reagents compared. Mouse cerebellum cryosections were labeled with secondary antibodies only (no primary antibody used) using 5 µg/mL of Alexa Fluor® 488 goat anti-mouse IgG (Cat. no. A11029), blocked with 6% bovine serum albumin, 5% normal goat serum, or BlockAid™ Blocking Solution (Cat. no. B10710), and compared against autofluorescence. Comparable fields were imaged with a 10X objective and the same exposure time. Intensity was measured using threshold measurement and plotted against relative fluorescence units.



## Cell and Tissue Blocking

The following protocol has been used for blocking a wide variety of samples, including cultured cells, cryosectioned or paraffin tissue sections, and zebrafish embryos. Host species or target species is not a concern for use with BlockAid™ Blocking Solution. Use BlockAid™ Blocking Solution undiluted or lightly-diluted (such as for diluting antibodies directly into the solution). All steps are typically performed at room temperature with gentle rotation.

**1.1** After the sample has been fixed and permeabilized, apply a sufficient volume of BlockAid™ Blocking Solution over the sample to completely immerse the sample. Allow at least 30 minutes for sufficient blocking of cultured cells, or at least 60 minutes for tissue sections or whole mounts.

**1.2** Dilute primary antibody into BlockAid™ Blocking Solution at a concentration or dilution recommended by the antibody provider, and apply the diluted antibody to the sample. Allow appropriate incubation time as prescribed by the antibody provider.

**1.3** Wash the sample well in PBS or other suitable physiological buffer solution.

**1.4** Dilute the secondary antibody directly into BlockAid™ Blocking Solution at a concentration or dilution recommended by the antibody provider, and apply the diluted antibody to the sample. Allow appropriate incubation time as prescribed by the antibody provider.

**1.5** Wash the sample well, counterstain if needed, and mount it in appropriate antifade mounting medium, such as the ProLong® Gold Antifade Reagent (Cat. no. P36930).

## Microsphere Protein Conjugate Blocking

BlockAid™ Blocking Solution is useful for applications in which streptavidin-, NeutrAvidin®-, biotin-, or protein A–labeled FluoSpheres® microspheres are employed as secondary detection reagents. For example, cells can first be exposed to a biotinylated antibody that is specific for an antigen of interest and then labeled with streptavidin- or NeutrAvidin®-coated FluoSpheres® microspheres. Alternatively, a biotinylated receptor ligand, such as epidermal growth factor, can be used as the primary detection reagent. The following protocol, designed for use with flow cytometry, describes a typical procedure for labeling cell-surface antigens of live cells using fluorescent microspheres as the secondary detection reagent.

### Bead Treatment

**2.1** Mix 1–5 µL of the microsphere suspension (0.5–1% solids in the case of Molecular Probes®' streptavidin-, NeutrAvidin®-, biotin or protein A–labeled FluoSpheres® microspheres) with 200 µL of BlockAid™ Blocking Solution. The amount of microsphere suspension for optimal labeling should be empirically determined for each application.

**2.2** Sonicate for ~5 minutes in an ultrasonic waterbath.

## Cell-Staining Procedure for Blocked Microsphere Protein Conjugates

**2.3** Prepare a single cell suspension (~$1 \times 10^6$ cells/mL) in PBS containing 1% bovine serum albumin (BSA).

**2.4** In a 15-mL centrifuge tube, incubate approximately 1 mL of the cell suspension (~$1 \times 10^6$ cells) with the desired primary antibody (or receptor ligand) for 30 minutes on ice. The optimal amount of primary probe should be empirically determined.

**2.5** Wash the cells to remove the primary detection reagent by filling the tube with PBS containing 1% BSA, gently mixing, pelleting the cells by centrifugation, and then removing the supernatant. Repeat.

**2.6** Add the entire amount of the microsphere–blocking solution suspension (prepared in **Bead Treatment**, page 3) to the primary antibody–labeled cells. Pipet up and down to resuspend the cells.

**2.7** Incubate the mixture on ice for 30 minutes.

**2.8** Wash the cells to remove the excess microspheres, as described in step 2.6.

**2.9** Add PBS containing 1% BSA to give the desired final volume (generally, ≤ to 0.5 mL). Pipet up and down to resuspend the cells.

**2.10** Analyze by flow cytometry or fluorescence microscopy using standard procedures.

## Product List

Current prices may be obtained from our website or from our Customer Service Department.

| Cat. no. | Product Name | Unit Size |
|---|---|---|
| B10710 | BlockAid™ blocking solution *for use with microspheres* | 50 mL |

# Purchaser Notification

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