MAXpack™ Immunostaining Media Kit
MAXblock™ Blocking Medium, MAXbind™ Staining Medium & MAXwash™ Washing Medium

Catalog Nos.: 15251 (MAXpack™ Immunostaining Media Kit)
15252 (MAXblock™ Blocking Medium)
15253 (MAXbind™ Staining Medium)
15254 (MAXwash™ Washing Medium)

Format: The MAXpack™ Immunostaining Media Kit contains one each of Catalog Nos. 15252-15254.
MAXblock contains 150 ml;
MAXbind contains 250 ml;
MAXwash contains 1000 ml

Applications: Immunofluorescence, Immunohistochemistry

Formulations: Please see the three attached Technical Data Sheets for MAXblock, MAXbind and MAXwash.

Storage: Store at 4°C. Guaranteed stable for 6 months when stored properly.

MAXblock, MAXbind & MAXwash are for *in vitro* research use only and are not intended for use in humans or animals.

MAXblock™ Blocking Medium

Catalog No.: 15252
Format: 150 ml
Applications: Immunofluorescence, Immunohistochemistry
Formulation: Non-mammalian blocking agent in PBS, pH 7.4, containing 0.09% sodium azide
Storage: Store at 4°C. Guaranteed stable for 6 months when stored properly.
Concentration: MAXblock is to be used at the delivered concentration (1X).

Description: MAXblock is a protein-based, non-mammalian blocking agent for use in immunofluorescence and immunodetection assays. Superior blocking is achieved utilizing a protein blend that demonstrates no cross-reactivity with secondary antibodies.

Quality Control: MAXblock has been tested for effectiveness in blocking non-specific binding of antibodies in immunofluorescence (see below) and immunohistochemistry.

Usage: For immunofluorescence, immunohistochemistry or immunocytochemistry, cells can either be grown directly on slides or coverslips, or spun down onto slides or coverslips. Blocking times are the same no matter which substrate the cells are on (see below).

Temperature	Blocking Time
4°C	Overnight
25°C	2 hours
37°C	1 hour

Coverslips can be placed cells side up in the well (one coverslip per well) of a six-well tissue culture plate. Use 1 ml MAXblock per well. Be sure to cover the plate to minimize evaporation.

Slides can be blocked cells side up in petri dishes or Coplin jars. (Glass jars are not recommended as protein adheres to glass). If Petri dishes are used, use enough MAXblock to completely cover the surface of the slide, and cover the dish during blocking to minimize evaporation. If blocking is performed at 25°C or 37°C, allow extra time to equilibrate the MAXblock to the proper temperature.

For antibody dilution and staining, it is strongly recommended that you use Active Motif's MAXbind™ Staining Medium (Cat. No. 15253). **Do not use MAXblock for diluting antibodies or for staining.**

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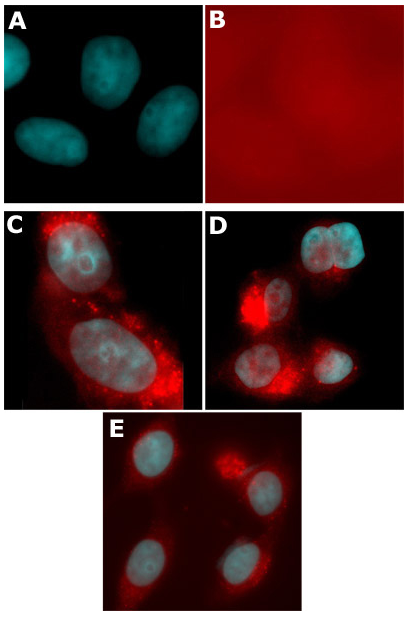

Figure 1: MAXblock Blocking Efficacy.
Methanol-fixed HeLa cells grown on a coverslip were blocked overnight in MAXblock (A), 5% non-fat dry milk (C), 5% BSA (D) or Thermo-Fisher Pierce Sea Block (E). Cells were then incubated with a fluorescent anti-rabbit secondary antibody at a dilution of 1:250 (far above the recommended dilution to demonstrate the effectiveness of MAXblock), washed with MAXwash and mounted using MAXfluor™ DAPI (Cat. No. 15257). Any observed signal (pseudo-colored red) is caused by non-specific binding of the secondary due to incomplete blocking. Note that no staining is observed in panel A, demonstrating the effectiveness of MAXblock. (Panel B is a 150% overexposure of the fluorescent channel of the MAXblock slide, which shows cell position and demonstrates that antibody binding is barely detectable above background.) Experiments with an anti-mouse secondary gave nearly identical results.

MAXbind™ Staining Medium

Catalog No.: 15253
Format: 250 ml
Applications: Immunofluorescence, Immunohistochemistry, Western blotting & ELISA
Formulation: Non-mammalian antibody binding agent in PBS, pH 7.4, containing 0.1% Triton X-100 and 0.09% sodium azide
Storage: Store at 4°C. Guaranteed stable for 6 months when stored properly.
Concentration: MAXbind is to be used at the delivered concentration (1X).

Description: MAXbind Staining Medium is a non-mammalian incubation agent that optimizes antibody staining in immunofluorescence and immunostaining assays. Used in combination with MAXblock™ Blocking Medium (Cat. No. 15252), MAXbind promotes superior antibody binding while helping to eliminate non-specific primary and secondary antibody binding.

Quality Control: MAXbind was tested for effectiveness in promoting antibody binding in immunofluorescence (below) as well as in IHC

Instructions: For immunofluorescence, immunohistochemistry or immunocytochemistry, cells can be grown either directly on slides or coverslips, or spun down onto slides or coverslips. The volumes below assume cells grown on coverslips in the wells of a 6-well plate.

1. After blocking cells with MAXblock or similar for 1 hour in a humidified environment, *e.g.* an incubator or slide warmer, aspirate the MAXblock and add 1 ml 1X MAXwash™ Washing Medium (Cat. No. 15254), or similar. Rock the plate for 10 minutes on a rotating platform. During this wash step, dilute your primary antibody to an appropriate dilution in MAXbind. For a coverslip in the well of a 6-well plate, you will need to add 1 ml. To make a 1:500 dilution, dilute 2 µl primary antibody in 1 ml MAXbind per well.
2. Aspirate the MAXwash and add 1 ml of the diluted primary antibody to each well. Incubate for 1 hour at 37°C in a humid environment, *e.g.* an incubator or slide warmer.
3. Aspirate the diluted primary antibody from the cells, add 1 ml 1X MAXwash and rock the plate for 10 minutes on a rotating platform. Aspirate the MAXwash and repeat 2 more times for a total of 3 washes. During the last wash, dilute your secondary antibody in MAXbind to a dilution of 1:500 to 1:2000. You will need to add 1 ml of diluted secondary antibody per well.
4. Incubate the diluted secondary antibody for 1 hour at 37°C in a **darkened**, humid environment, *e.g.* an incubator or slide warmer. From here forward, it is important to limit the amount of light exposure to the fluorescent dye on the secondary antibody.
5. Aspirate the diluted secondary antibody from the cells and add 1 ml 1X MAXwash. Rock the plate for 10 minutes on a rotating platform. Aspirate the MAXwash and repeat 3 more times for a total of 4 washes.
6. After the last wash, carefully remove the coverslip from the well using flat-edged forceps. In some cases, the coverslip is slightly stuck to the bottom of the well and may need to be dislodged using a beveled needle. Using the needle, carefully stand up the coverslip in the well and grab it with forceps.
7. Dry the coverslip to remove excess MAXwash. Hold the corner of the coverslip to a Kimwipe to remove any excess MAXwash from the coverslip. Remember to limit light exposure. You are now ready for counterstaining and slide mounting.

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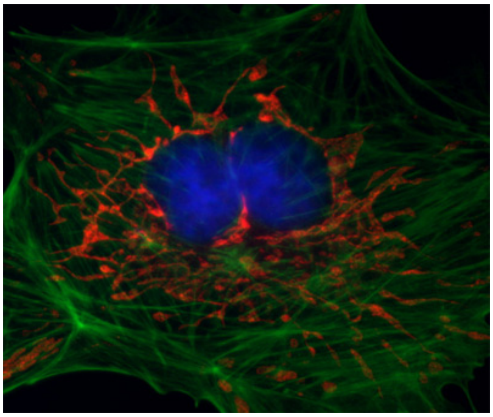

Immunofluorescence staining with MAXbind.
Bovine pulmonary artery endothelial cells were labeled for mitochondria (red), F-actin (green) and DAPI (blue) using MAX Stain™ immunofluorescence and immunostaining products. In addition to MAXbind, MAXblock, MAXwash and MAXfluor™ DAPI were used in the creation of this image.

MAXwash™ Washing Medium

Catalog No.: 15254
Format: 1000 ml
Applications: Immunofluorescence, Immunohistochemistry, Western blotting & ELISA
Formulation: Proprietary washing buffer containing 0.001% sodium azide.
Storage: Store at 4°C. Guaranteed stable for 6 months when stored properly.
Concentration: MAXwash is to be used at the delivered concentration (1X).

Description: MAXwash is a proprietary formulation designed to eliminate non-specific primary and secondary antibody binding.

Quality Control: MAXwash was tested in immunofluorescence for effectiveness in eliminating off-target antibody binding.

Instructions: MAXwash is to be used at the concentration supplied for washing cells after fixation and blocking steps, as well as incubations with primary and secondary antibodies. The amount needed will depend upon the type of tissue culture plate you are using. If you are using 6-well plates, 1 ml 1X MAXwash is used per well for each wash step.

Typically, one wash is performed after fixing cells with methanol and blocking with MAXblock™ Blocking Medium (Cat. No. 15252), or similar. Three washes are usually performed following incubation with primary antibody diluted in MAXbind™ Staining Medium (Cat. No. 15253), or similar. Four washes are usually performed following incubation with diluted secondary antibody.

Each wash step is generally performed for 10 minutes on a rocking platform. The optimal number and duration of wash steps may need to be adjusted depending upon your cell line, primary and secondary antibodies.

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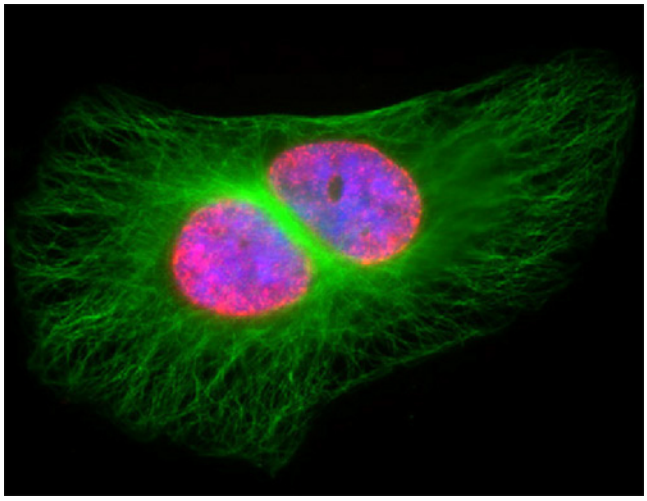

Immunofluorescence staining with MAX Stain™ reagents.
HeLa cells were stained using the MAX Stain line of products for immunofluorescence and immunostaining. Green: alpha Tubulin Mouse mAb (Clone 5-B-1-2) (Cat. No. 39527).
Red: Histone H3 trimethyl Lys27 Rabbit pAb (Cat. No. 39155). Blue: DAPI.
In addition to MAXwash, MAXblock, MAXbind™ Staining Medium (Cat. No. 15253) and MAXfluor™ DAPI Mounting Medium (Cat. No. 15257) were used in the creation of this image.