INNOVEX BIOSCIENCES

# Background Buster

***Peptide Blocker for Removal of background staining***

## FOR USE with HUMAN AND ANIMAL TISSUE STAINING

***For IHC, Fluorescence, in situ probes and Flow cytometry***

## *Technical Data Sheet*

**Reagent Category**

**Monoclonal primary antibody to Ki-67 antigen**

**ITEM#: IMI01172E (50ml)**
**ITEM#: IMI01177E (125ml)**

**Antibody Formats Supplied**

**□ 50 ml of Background Buster working solution, Ready-To-Use**

**□ 125 ml of Background Buster working solution, Ready-To-Use**

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### INTRODUCTION

Background staining or non-specific staining is an often-encountered problem in immunohistochemistry, in immunofluorescence and in situ stains. Background staining is caused by a number of factors such as cross reactivity of antibodies with the shared epitopes in the tissue, by the presence of natural and/or contaminating antibodies present in the primary antibody and/or the secondary antibody, by ionic interactions, by the presence of carbohydrates and by endogenous biotin present in the tissue. Eradicating background is most important for obtaining background-free specific staining for the ease of qualitative and quantitative evaluation.

### PRODUCT DESCRIPTION

**Innovex Background Buster** is a recombinant protein Blocker that eradicates all general background staining. Background Buster removes all background staining caused by primary antibodies, by the staining reagents, by the chromogens, by the fixatives and by endogeneouse biotin present in tissues such as liver and spleen and kidney. Background Buster is used in place of all normal sera and other blocking solution for removing background staining in both human and animal tissues**.**

**Innovex Background Buster** is applicable IHC staining, to immunofluorescence staining and to in situ probe staining in both human and animal tissues. It is also applicable to flow cytometric assay.

**Innovex Background Buster** is a must for animal tissue staining. It is especially essential when staining mouse antibodies on mouse tissues (Mouse-on-Mouse) and other staining of identical antibodies on identical species tissues e.g. Rabbit-on- Rabbit, Rat –On-Mouse, Mouse-on- Rat or Goat-on-Goat, etc. A 30-minute incubation with Innovex Background Buster is recommended prior to the application of the primary antibody for staining of identical species primary antibodies and tissues.

It should be noted that in immunoperoxidase-IHC staining, another type of background and non-specific staining is caused by red blood cell staining; this is due to endogenous peroxidase enzyme present in red blood cells. This type of background requires a pre-treatment step with 3% hydrogen peroxide (H2O2) in water; this blocking step should precede the blocking step with **Innovex Background Buster.**

### APPLICATION/INTENDED USE

**"Background Buster"** is intended for blocking non-specific binding and eradicating background in IHC, immunofluorescence and *in situ* probe stains for both human and animal tissues.

### STORAGE CONDITIONS

Store in refrigerator at 2-8o.C through the expiration date noted on the vial label.

### PRODUCT FORMAT

Working solution (Ready-To-Use), no dilution or adjustments required.

## INSTRUCTIONS

### Specimen Preparation for IHC Staining

***For paraffin sections:*** Deparaffinize sections and rehydrate in water. ***For Frozen sections:*** Cut sections, dry and fix in cold acetone or the fixative of choice. Incubate in PBS for 3 minutes at room temperature. ***For Cytocentrifuge preparations:*** Prepare cytocentrifuge preparations of cell suspensions and observe the following instructions:

**1.** When using peroxidase enzyme conjugate label (staining with DAB or AEC), quench tissue endogenous peroxidase activity by immersing slides in 3% $H_2O_2$ in water and incubate for 10 minutes. Rinse with water.

**2.** Apply 2-3 drops of "Background Buster" to achieve specimen coverage.

**3.** Incubate for 10 minutes at room temperature for human tissue. For ANIMAL TISSUES incubate for 20 minutes prior to the application of the primary antibody. For MOUSE-TO-MOUSE, Mouse-To-Rat, Rat-To-Rat staining incubate for 30 minutes prior to application of the primary antibody.

**4.** Rinse water and proceed with enzyme immunostaining or immunofluorescence or in-situ probe staining by following the manufacturer's instruction.

**For removal of endogenous biotin**

Innovex Background Buster can be used for blocking endogenous biotin in place of avidin block or egg white. Tissues that are rich in biotin include kidney, liver and spleen.

*Apply 2-3 drops of Innovex Background Buster to achieve specimen coverage and Incubate for 10 minutes at room temperature for both human and animal tissues. the application of the primary antibody. For MOUSE-TO-MOUSE, Mouse-To-Rat, Rat-To-Rat staining incubate for 30 minutes prior to application of the primary antibody.*

**5.** Rinse water and proceed with enzyme immunostaining or immunofluorescence or in-situ probe staining by following the manufacturer's instruction.

**BACKGROUND BUSTER IS A MUST FOR ANIMAL TISSUE STAINING.**

**For ANIMAL TISSUE STAINING background removal**

**"Background Buster" is a must for animal tissue staining, it removes all background generated by cross reactivity of primary antibodies with animal tissues.**

**1.** Apply 2-3 drops of "Background Buster" to achieve specimen coverage prior to the application of the primary antibody.

**2.** Incubate for 20 minutes at room temperature, rinse with water and apply the primary antibody

**3.** Proceed with immunostaining per staining kit instruction.

**For *in-situ* stains**

Apply "Background Buster" post hybridization and prior to the application of conjugated secondary antibody. Incubate for 10 minutes.

***For Immunofluorescence staining of tissues and cytosmears***

Following the specimen preparation:

1. Treat sections or smears with enough number of drops (3 to 6) of "Background Buster" to achieve specimen coverage.
2. Incubate for 10 minutes at room temperature.
3. Rinse in appropriate wash buffer and proceed with application of fluorochrome conjugated antibody (direct method) or with the application of non-conjugated primary antibody, followed by fluorochrome conjugated secondary antibody (indirect method).

***For Flow cytometric test samples***

Test specimen consisting of blood cells or tumor cell suspension are treated as follows:

1. Incubate cell suspensions with "Background Buster" in a test tube or in a microtiter plate with 0.2 ml/106 cells.
2. Incubate for 5-10 minutes.
3. Wash with the appropriate assay wash buffer and proceed with application of the conjugated (direct method) or unconjugated primary antibody followed by fluorochrome conjugated secondary antibody (indirect method).

***FOR IN VITRO RESEARCH USE ONLY***

**FOR ADDITIONAL TECHNICAL SUPPORT:**

American MasterTech

scientific laboratory supplies

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