INNOVEX biosciences

Background Buster

Peptide Blocker for Removal of background staining

FOR USE with HUMAN AND ANIMAL TISSUE STAINING

For IHC, immunofluorescence (IF), *in situ* probes and Flow cytometry

Technical Data Sheet

Reagent Category

Peptide Blocking Agent for Background removal

Specific Reagents Supplied

- ☐ **50 ml of Background Buster working Solution, Ready-To-Use**
- ☐ **125 ml of Background Buster working Solution, Ready-To-Use**
- ☐ **7ml of Background Buster working Solution, Ready-To-Use**

PRODUCT # NB306-50 (50 ml), NB306 (125 ml), NB306-7 (7ml)

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INTRODUCTION

Background staining or non-specific staining is an often-encountered problem in immunohistochemistry (IHC) , in immunofluorescence labeling (IF) 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 peptide Blocker that eradicates all general background staining. **Background Buster removes all background staining caused by** primary antibodies, by secondary staining reagents, by chromogens, by fixatives, by high heat retrieval (HIER) and by endogeneouse biotin present in tissues such as liver, pleen and kidney. **Background Buster is** used in place of normal sera and other blocking solution for removing background staining in both human and animal tissues.

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

Innovex Background Buster is a must for animal tissue staining, it is especially essential when staining identical species tissue and antibodies such as mouse antibodies on mouse tissues (Mouse-on-Mouse) and Rabbit-on- Rabbit. 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 such as mous-on-mouse.
The use of Background Buster is highly recommended for staining of indirect species (non-identical species tissue/ antibody) such as Rat –On-Mouse, Mouse-on- Rat, Mouse-on-Rabbit, etc. A 20-minute incubation with Innovex Background Buster is recommended prior to the application of the primary antibody for staining of Indirect (non-identical species primary antibodies and tissues).
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% freshly made hydrogen peroxide (H2O2) in water or use of INNOVEX STABLE PEROXIDE BLOCK. this blocking step should precede the blocking step with **Innovex Background Buster.**

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APPLICATION/INTENDED USE

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

STORAGE CONDITIONS

Store in refrigerator at 2-8^{o}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 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 DI water OR in INNOVEX PEROXIDE BLOCK and incubate for 10 minutes. Rinse with water.
2. Apply 2-4 drops of "Background Buster" to achieve specimen coverage.
3. Incubate for 10 minutes at room temperature for human tissues. For Indirect species antibody and ANIMAL TISSUES incubate for 20 minutes prior to the application of the primary antibody. For identical species tissue and antibody such as Mouse-On-Mouse, Mouse-on-Rat, Rat-on-Rat; incubate for 30 minutes prior to application of the primary antibody.
   For excessive general background staining or background staining due to endogenous biotin; Incubate for 30 minutes.
4. Rinse with water and proceed with IHC staining or immunofluorescence labeling 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 30 minutes at room temperature for both human and animal tissues prior to the application of the primary antibody.
Rinse in water and proceed with enzyme immunostaining or immunofluorescence labeling 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 staining 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. For Indirect species antibody and tissue such as mouse-on-rabbit; incubate for 20 minutes prior to the application of the primary antibody. For identical species tissue and antibody such as Mouse-On-Mouse, Mouse-on-Rat, Rat-on-Rat; incubate for 30 minutes prior to application of 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 labeling 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 –15 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/10^6 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 RESEARCH USE ONLY

FOR ADDITIONAL TECHNICAL SUPPORT
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