American MasterTech
scientific laboratory supplies
POST OFFICE BOX 2539 LODI, CALIFORNIA 95241 TELEPHONE: 1 (800) 860 4073 FACSIMILE: 1 (209) 368 4136

INNOVEX BIOSCIENCES

Histo-STAT™ Alkaline Phosphate System *w/ Fast Red*

No-Wash, 2-STEP Rapid Immunostaining System

Background Free, 30 minutes, 10 minutes STAT Immunostaining System

Technical Data Sheet

Specific Reagents Supplied (500-800 Slides)
□ **50 ml of Second Step Reagent**
□ **30 ml of Fast Red**

PRODUCT# IMI03008E (Alkaline Phosphate System w/ Fast Red)

INTRODUCTION

Immunostaining detection systems are used to determine the presence, localization and the density of cellular and tissue antigens in building assays. In immunohisto/cytochemistry and in ELISA procedures antigens are either visualized or measured by enzyme immunochemical assays.

PRODUCT DESCRIPTION

HISTO-STAT Rapid Immunostaining System is a 2-step immunostaining system engineered for high sensitivity and short incubation periods. This detection system includes two components, the alkaline phosphatase Second Step Reagent and FAST RED. This staining system is universally applicable to all mouse and rabbit primary antibodies. This system is also applicable to tissues and cell specimens of all species source. This system is further applicable to all tissues and cells regardless of their method of processing, e.g. paraffin sections, cryostat sections, cytocentrifuge preparations or cell smears.

HISTO-STAT Rapid Immunostaining System is an ideal staining system for staining of primary antibodies in both paraffin and frozen sections. It is free of biotin, avidin and strepavidin. The use of this staining system offers sensitivity and speed as well as eliminating the need for biotin blocking step in tissues or cells that are rich in endogenous biotin, **its use offers further speed by elimination a reagent incubation step and a wash step.**

INTENDED USE/APPLICATION

This product is intended for immunolocalization of mouse or rabbit primary antibodies in tissue and cell smears in immunohisto/cytochemical staining and in ELISA procedures.

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SYSTEM COMPONENT SPECIFICATIONS

Recommended Incubation times for the systems are:

- Primary antibodies (not provided): 30 minutes for paraffin sections, 5-10 minutes for frozen sections and cell smears.
- Alkaline phosphatase Second Step Reagent: 30 minutes for paraffin sections, 10 minutes for frozen sections and cell smears.
- Fast Red substrate/ chromogen mixed solution: 10-15 minutes.

INSTRUCTIONS

For Paraffin slides: Deparaffinize slides and rinse in water.
For Frozen sections: Cut frozen sections, fix with fixative of choice.

1. Incubate the section or smear with the primary antibody of choice (not provided) for 30 minutes for paraffin sections, 5-10 minutes for frozen sections and cell smears
2. Rinse with PBS for 10 seconds. For best and strong staining rinse with Innovex Alk-phos Enhancing wash buffer (Product # IMI00592E).
3. Incubate with HISTO-STAT Second Step Reagent for 30 minutes for paraffin sections and 10 minutes for frozen sections.
4. Rinse for 10 seconds.

Substrate/chromogen mixing protocol

When using Fast Red chromogen, mix prior to use by dissolving one Fast Red tablet to 5 ml of Ready-to-use substrate buffer in the provided graduated mixing tube.

1. Incubate with mixed Fast Red/substrate for **10 minutes**.
2. Rinse in water.
3. Counterstain with an aqueous based hematoxylin when using Fast Red chromogen, mount with permanent mounting media. Do not mount with xylene or toluene based mounting media.

STORAGE CONDITIONS

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

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INNOVEX BIOSCIENCES

Polymer HRP Histo-STAT™

No-Wash, 2-STEP Rapid Immuno-peroxidase staining of MOUSE and RABBIT primary antibodies

(Biotin Free)

Technical Data Sheet

Specific Reagents Supplied (500-800 Slides)

□ 50 ml of HISTO-STAT, 2 STEP Peroxidase Staining System

PRODUCT# IMI03002E (AEC Kit) | PRODUCT# IMI03004E (DAB Kit)

PRODUCT DESCRIPTION

POLYMER HISTO-STAT, 2 Step Staining system is **a No wash, No Background** staining system formulated for 2-step immunostaining of primary antibodies raised in mouse and rabbit. The **POLYMER, HISTO-STAT, 2-Step** is bio-engineered for high sensitivity and short incubation periods. Innovex HISTO-STAT staining (detection) system includes two components only, a Multivalent anti rabbit and anti mouse Polymer Second Step- Peroxidase Reagent and a short incubation and stable AEC OR DAB substrate/ chromogens. This component of Polymer HISTO-STAT staining system is universally applicable to all mouse and rabbit primary antibodies. This system is also applicable to all species tissues and cell specimens. This system is further applicable to all tissues and cells regardless of their method of processing, e.g., paraffin sections, cryostat sections, cytocentrifuge preparations or cell smears.

POLYMER HISTO-STAT is an ideal IHC staining system for staining of primary antibodies in both paraffin and frozen sections as well as cytosmears. HISTO-STAT staining system is free of biotin, avidin and streptavidin. The use of this staining system provides sensitivity and speed as well as eliminating the need for biotin blocking step in tissues or cells that are rich in endogenous biotin. The use of Polymer HISTO-STAT, 2 Step further provides speed by eliminating one reagent incubation step and one wash step. " POLYMER HISTO-STAT" Staining system is designed to eliminate the need for re-titration of primary antibodies upon the switch over to this system.

INTENDED USE/APPLICATION

This product is intended for immunolocalization of MOUSE and RABBIT primary antibodies in tissue and cell smears in immunohisto/cytochemical staining and in ELISA procedures.

INSTRUCTIONS

ALL INNOVEX PRODUCTS ARE DESIGNED TO BE IMPLEMENTED AT ROOM TEMPERATURE (NO HEAT IS REQUIRED).

No protein blocking and NO extensive washes are required when staining with Innovex staining systems

1. Following deparaffinization Quench endogenous peroxidase activity by immersing tissue slides in freshly made 3% hydrogen
2. Peroxide (H2o2) prepared in distilled water. This step is essential to eliminate red blood cell staining.
3. Rinse with tap water for 1 minute.

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4. Rinse with PBS or Innovex "Signal HRP Enhancing Wash Buffer" once for 5 seconds prior to application of primary antibody.
5. Incubate the section or smear for 20-30 minutes with mouse and rabbit primary antibodies (not provided).
6. Rinse with PBS or Innovex Signal HRP Enhancing Wash Buffer once for 5 seconds.
7. Incubate with POLYMER HISTO-STAT Second Step Reagent for 30 minutes for paraffin sections or 15 minutes for frozen sections
8. Rinse with PBS or Innovex Signal HRP Enhancing Wash Buffer once for 5 seconds.
9. Incubate with AEC/substrate solution for 10 minutes or DAB/substrate solution for 5 minutes.
10. Rinse with tap water.
11. Counterstain with hematoxylin
12. Mount slides as usual

Substrate/chromogen mixing protocol

- When using AEC chromogen, mix AEC by adding 2 drop of AEC chromogen (component 2) to 2 ml of Ready-to-use substrate buffer (component 1) in the provided graduated mixing tube. Mix by inversion. Left over, mixed AEC substrate/chromogen solution is stable for two weeks when kept refrigerated and can be re-used within 14 days. This minimizes the reagent waste and disposal costs and efforts.
- When using DAB chromogen mix DAB by adding 2 drops of DAB chromogen (component 2) to 2 ml of Ready-To-Use substrate buffer (component 1) in the provided graduated mixing tube. Mix by inversion. Left over, mixed DAB chromogen solution is stable for two weeks when kept refrigerated and can be re-used within 14 days. This minimizes reagent waste and disposal costs and efforts.

*Please note that a well-formulated, non-oxidized DAB should produce a brown stain, the formation of a very dark DAB stain is indicative of DAB oxidation and it causes background. Therefore, when darker DAB stain is desired, apply Innovex Room temperature Quick DAB Enhancer solution (product# NB308) following the final rinse step of DAB in tap water. Innovex DAB Enhancer can be applied before or after counterstaining for 1-5 minutes. This DAB enhancer does not cause any background.

STORAGE CONDITIONS

Store in refrigerator at 2-8^{o}C through expiration date noted on the vials.

IMPORTANT NOTES:

- Innovex STAT-Q (3 step) and HISTOSTAT polymer staining reagents and components are no wash, no background staining kits, a single 5-10 second washes in between incubation steps will be sufficient rinse.

- Innovex STAT-Q (3 step) and HISTOSTAT polymer staining kits and primary antibodies are free of background; they contain built -in recombinant protein blocking agents and do not require pre-protein blocking prior to the application of primary antibody.

- Innovex primary antibodies are engineered to perform at 10 minute for STAT-Q (3-step) and at 30 minutes for HISTO-STAT (2 step) staining systems. However, when using other manufacturer's primary antibodies and rinsing with Innovex Signal Enhancing Wash Buffers, decrease the recommended incubation time by half or dilute antibody by 2 folds and observe the original manufacturer's recommended incubation time.

- Innovex Staining systems and primary antibodies are engineered to be free of background, however, when background is encountered due to the nature of the tissue specimen, tissue processing and fixation or mix and matching of reagents from different sources, the application of Background Buster (product # IMI01172E) for non-lymphoid tissues and Fc Receptor Blocker (product# IMI01565E) for lymphoid tissues is highly recommended for eradication of background staining.