



HISTO-STAT™ RABBIT- Alkaline phosphatase Second Step Reagent **For No-Wash, 2-Step Rapid Immuno-alkaline phosphatase staining of** **RABBIT primary antibodies** **(Background Free)**

Product # NB317R

125 ml of Rabbit Alkaline Phosphatase Second Step Reagent (500-600 tests)

INTRODUCTION

Immunostaining detection systems are used to determine the presence, localization and density of cellular and tissue antigens in immuno-binding assays. In immunohistochemistry and in ELISA procedures antigens are either visualized or measured by enzyme immunochemical assays.

PRODUCT DESCRIPTION

Innovex HISTO-STAT-RABBIT, alkaline phosphatase Second Step reagent is a **no-wash, no-background staining reagent formulated for 2- step** immunostaining of primary antibodies that are raised in rabbit. The HISTO-STAT Second Step-alkaline phosphatase reagent is engineered for high sensitivity and short incubation period. Innovex HISTO-STAT, alkaline phosphatase Second Step reagent is universally applicable to all 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 and cell smears.

“HISTO-STAT” is an ideal staining system for staining of rabbit primary antibodies for 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. **Its use further provides speed by eliminating one reagent incubation step and one wash step.** **“HISTO-STAT” staining system is designed to eliminate the need for re-titration of primary antibodies upon the switch over to this system.**

APPLICATION / INTENDED USE

This product is intended for staining of RABBIT primary antibodies in tissue and cell smears in immunohistochemical staining and in ELISA procedures.

SYSTEM COMPONENTS and SPECIFICATIONS

Recommended incubation times for the system components are: RABBIT primary antibodies (not provided): Observe manufacturers recommended incubation time. When using Innovex primary antibodies, do not alter incubation times as they are engineered to perform optimally with PBS, Tris buffer or Signal Enhancing wash buffers at **30** for HISTO-STAT (2 step) staining system.

- Stabilized alkaline phosphatase goat anti Rabbit Second Step Reagent: **30 Minutes** incubation for paraffin sections and **20 minutes** incubation for frozen sections.

CONTINUED OVER

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. Rinse in PBS or Innovex "Signal HRP Enhancing Wash Buffer" once for 5 seconds prior to application of primary antibody.
2. Incubate the section or smear for **30** minutes with rabbit primary antibodies (not provided), when using other manufacturer's primary antibodies observe the manufacturer's recommended incubation time.
3. Rinse with PBS or Innovex Signal HRP Enhancing Wash Buffer once for 5 seconds.
4. Incubate with HISTO-STAT, **alkaline phosphatase - Rabbit Second Step Reagent** for **30 minutes** for paraffin sections or **20 minutes** for frozen sections
5. Rinse with PBS or Innovex Signal HRP Enhancing Wash Buffer once for 5 seconds.
6. Incubate with Innovex Fast Red substrate/ Chromogen for 10 minutes or with Innovex Brown for 15 minutes. **When using other manufacturer's chromogens for alkaline phosphatase observe the manufacturer's recommended incubation time.**
7. Rinse in tap water.
8. Counterstain with hematoxylin (Innovex Product # NB305).
9. Mount slides as usual or with Innovex non-xylene based permanent "Advantage Mounting Media" Product #NB300). When using substrate/ chromogens that are soluble in alcohol or xylene, DO NOT mount with xylene based mounting media.

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.

Innovex Brown chromogen is supplied in a single component, No mixing required. Simply apply.

1. Incubate with mixed Fast Red/substrate for **10 minutes** or with Innovex Brown chromogen for **15 minutes**.
2. Rinse in water.
3. Counterstain with an aqueous based hematoxylin (Innovex Product #NB305) when using Fast Red chromogen, mount with Innovex Advantage permanent mounting media. Do not mount with xylene or toluene based mounting media.

OR

4. Innovex Brown is permanent and can be mounted from alcohol and xylene. Innovex Brown stained slides can be mounted with Innovex Advantage permanent Mounting Media (Innovex Product #NB300) or can also be mounted with xylene or toluene based mounting media.

STORAGE CONDITIONS

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

CONTINUED OVER

Important Notes:

- **Innovex HISTOSTAT (2-step) 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 HISTOSTAT (2-step) 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 when staining human tissue. For animal tissue IHC staining blocking with Innovex Background Buster (Recombinant protein blocker) prior to the application of the primary antibody is essential**
- **Innovex primary antibodies are engineered to perform at 20 minutes for HISTO-STAT (2 step) staining systems. However, when using other manufacturer's primary antibodies observe the 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 # NB306) for non-lymphoid tissues and Fc Receptor Blocker (product# NB309) for lymphoid tissues is highly recommended for eradication of background staining.**

**FOR ADDITIONAL TECHNICAL SUPPORT
CALL: 1.800.622.7808 US and Canada
Phone: (510) 234-6600 Web: innvx.com**

