HISTO-STAT 1-Step POLYMER Multivalent AEC Staining System

***For* rapid IHC & ICC staining of MOUSE, RABBIT, RAT primary antibodies**

Background-Free, Biotin-Free

Large volume kit: Product number: NB312LC (50 ml kit)
Small volume kit: Product number: NB312LC-20 (20 ml kit)

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PRODUCT DESCRIPTION

1-step Polymer HISTO-STAT Staining system is a No-wash, No-Background staining system formulated for 2-step IHC & ICC staining of mouse, rat and rabbit primary antibodies. 1-Step POLYMER, HISTO-STAT system is bio-engineered for high sensitivity, short incubation periods and lack of background.

Innovex HISTO-STAT Polymer Staining (detection) system includes ancillary reagents and two main staining components; A multivalent anti Rabbit, anti Mouse, anti Rat HRP Polymer and stable AEC or DAB substrate/ chromogens. This **1-step Polymer staining system** is universally applicable to staining all mouse, rat and rabbit primary antibodies. This system is also applicable to IHC and ICC staining of both human and animal tissues. This system is universally applicable to staining all tissues and cells regardless of processing methods, e.g., paraffin sections, cryostat sections, cytocentrifuge preparations and cell smears.

"HISTO-STAT" 1-Step Polymer is an ideal system for staining primary antibodies on paraffin and frozen tissue sections and on cytosmears and cytospins. This 1-Step Polymer system is free of biotin, avidin and streptavidin and its use provide sensitivity and speed as well as eliminating the need for biotin blocking step for tissues that are rich in endogenous biotin. The use of **HISTO-STAT, 1-Step Polymer 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 IHC and ICC staining of MOUSE, RAT and RABBIT primary antibodies in tissues and cell preparations.

SYSTEM COMPONENTS and SPECIFICATION

- 60 ml of Perox-Block: 15-minute incubation.
- 50 ml of Background Buster: 10-minute incubation
- MOUSE or RABBIT or RAT primary antibody ***(not provided);*** 1-hour incubation**; Observe manufacturer recommended incubation time for the primary antibodies employed.**
- 50 ml of Histo-Stat,1-Step HRP Polymer: 45-minute incubation.
- 70 ml of stable two-component AEC substrate/chromogen: 20-minute incubation.
- AEC Enhancer: 5-minute incubation, **this step is optional.**

INSTRUCTIONS

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

***No serum blocking and no extensive washes are required when staining with HISTO-STAT 1-Step Polymer* system.**

DO NOT Rinse with Tris buffer.

Following deparaffinization; Quench endogenous peroxidase enzyme activity by applying PEROX-BLOCK for **15-minutes** (provided in the kit) and then rinse 2 times with water for **30-seconds** each time.

1. Retrieve paraffin sections with Innovex Uni-Trieve OR with Retrieval of choice.
2. Apply Background Buster for **10-minutes** (provided in the kit).
3. Rinse with PBS or Innovex HRP Enhancing Wash Buffer for **10-seconds.**
4. Incubate the section for **1-hour** with Mouse or Rat or Rabbit primary antibody (not provided); **Observe manufacturer recommended incubation time for the primary antibody employed.**
5. Rinse with PBS or HRP Enhancing Wash Buffer once for **10-seconds.**

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6. Incubate with the **HISTO-STAT, 1-step HRP Polymer** for **45-minutes** for paraffin and frozen sections.
7. Rinse with PBS or HRP Enhancing Wash Buffer for **10-seconds.**
8. Incubate with mixed AEC substrate/chromogen solution for **20-minutes** and rinse with water.
9. Apply AEC Enhancer for 5 minutes and rinse with water (provided in the kit); **This step is optional.**
10. Counterstain with water-based hematoxylin (Innovex Aqua Hematoxylin, product number NB305) or other water-based hematoxylin. **Do not counterstain with hematoxylin containing alcohol, AEC dissolves in alcohol.**
11. Rinse hematoxylin with water by filling and emptying the slide holder vessel with water until water runs clear.
12. Mount slides from water with Innovex permanent "Advantage Mounting Media" (product number NB300). Air dry slides before mounting with Advantage Mounting media.
    Do not mount AEC-stained slides with xylene or toluene based mounting media as AEC dissolves in organic solvents such as alcohol, xylene and toluene.

AEC Substrate/chromogen mixing protocol

Mix AEC substrate and chromogen by adding 1 drop of AEC chromogen (component 2) **to 1 ml of substrate buffer** (component 1) in the provided graduated mixing tube or in a clean tube of choice. Mix gently and thoroughly by drawing the mixture in and out of transfer pipette a few times.
Un-used **mixed AEC solution is stable for 1-week when kept refrigerated and can be re-used within 7 days post initial mixing, this minimizes reagent waste and cost.**

STORAGE CONDITIONS

Store in refrigerator at 2-8ºC through expiration date noted on the kit.

Important Notes:

- **HISTO-STAT 1-Step Polymer staining kits and reagents are minimal wash, a single 10-second wash in between incubation steps is sufficient. Longer washes do not adversely affect the results.**
- **HISTO-STAT 1-Step Polymer kit is universally applicable to staining all antibodies specified in this data sheet; Follow manufacturer recommended incubation time for primary antibody employed.**
- **Rinsing with Innovex Signal Enhancing Wash Buffer amplifies staining signal and produces sharper and more resolved staining.**
- **Do not rinse with tris buffer.**

FOR PROFESSIONAL AND RESEARCH USE

FOR ADDITIONAL TECHNICAL SUPPORT
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