

STAT Animal IHC Kit Universal Animal IHC staining Kit

For Background Free, Rapid IHC staining of Mouse-On-Mouse, Xenograft and IHC staining of All-species animal tissues

Product number: 329ANK-20 (with DAB); 200 plus slide kit

Product number: 329ANK-60 (with DAB); 800 plus slide kit

INTRODUCTION

Immunostaining staining kits are used to determine the presence, localization and density of antigens in binding assays. In immunohistochemistry (IHC) antigens are visualized by enzyme immunochemical kits also known as detection or staining kits. IHC staining kits usually consist of a secondary antibody and a third step reagent of an enzyme such as alkaline phosphatase or horseradish peroxidase (HRP) conjugated to a matrix. The enzyme is then incubated for a short time with its appropriate substrate and chromogenic substance for color development. The rate of color development measures the enzyme concentration by qualitative (IHC), semi quantitative (image analysis).

PRODUCT DESCRIPTION

STAT Animal IHC kit is a background-free, minimal –wash IHC staining kit bio-engineered for No-Background, rapid 10 second washes and short incubation steps. This staining kit is universally applicable to staining mouse-on-mouse, Xenograft staining and staining of all animal tissues and all Animal cell preparations (cyto smears, cytocentrifuge preps).

STAT animal IHC kit is applicable to staining all Mouse, Rabbit & Rat primary antibodies on all species of animal tissues and cell preparations. This staining kit is suitable for staining All Animal Species tissues and cells regardless of their method of processing (e.g., paraffin sections, cryostat sections, cytocentrifuge preparations and cell smears).

STAT animal IHC stains Mouse, Rat and Rabbit primary antibodies on all species animal tissues. This kit is designed to be virtually free of background when used with Background Buster (provided in kit). This Animal kit does NOT require serum or protein Blocking, however, a 30-minute blocking step with Innovex Background Buster prior to application of primary antibody is required.

STAT Animal IHC kit is also designed to eliminate the need for re-titration of primary antibodies when switching over to this system. When employing this kit no adjustment of currently employed primary antibody dilutions are necessary. Simply replace **STAT Animal kit** in place of the current detection system using the same primary antibody titers.

APPLICATION / INTENDED USE

This product is intended for IHC staining of mouse, rabbit and rat primary antibodies in all-species animal tissues, and also for IHC staining of identical species tissues/ antibodies such as Mouse-on-Mouse and Xenograft staining.

SYSTEM COMPONENTS and SPECIFICATIONS

Recommended incubation times for STAT Animal staining kit are:

- Background Buster: **30-minutes** (required).
- Mouse or Rabbit or Rat primary antibody (not provided): **30 -minutes to 1- hour**. Observe manufacturer's recommended incubation time for each primary antibody employed.
- Secondary Linking Antibody: **10-minutes**.
Peroxidase (HRP) enzyme: **10-minutes**.
- Liquid DAB chromogen: **5-minutes**.
- Fc Receptor Blocker; Required for Xenograft, spleen, liver, lymphoid tissues, CD markers, melanomas and tumor tissues and cells expressing Fc receptors.

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INSTRUCTIONS

30-minute blocking with Innovex Background Buster is required for staining Mouse-on-Mouse and animal tissues IHC staining (provided in the kit); DO NOT use other protein Blocks or serum blocks with animal tissues.

Following Deparaffinization and retrieval for paraffin sections;

1. Quench endogenous peroxidase activity by dropping on or immersing tissue slides for 15 minutes in INNOVEX STABLE PEROXIDE BLOCK or by immersing in freshly made 3% hydrogen peroxide (H₂O₂) prepared in DI water. This step is essential for eliminating red blood cell staining.
2. Rinse with water for by filling and emptying the jar 3 times with tap water.
3. Apply Background Buster for **30-minutes**.
4. Rinse with PBS or INNOVEX Enhancing Wash Buffer for **10-seconds**.
5. Incubate with primary antibody for **30-minutes to 1-hour** (observe vendor's recommended incubation time for primary antibody).
6. Rinse with PBS or INNOVEX Enhancing Wash Buffer for **10-seconds**.
7. Incubate with the Secondary Linking Antibody for **10-minutes**.
8. Rinse with PBS or INNOVEX Enhancing Wash Buffer for **10-seconds**.
9. Incubate with Peroxidase (HRP) Enzyme for **10-minutes**.
10. Rinse with PBS or HRP-Enhancing Wash Buffer for **10-seconds**.
11. Incubate with mixed DAB/substrate solution for **5-minutes**; DAB produces brown stain.
12. Rinse DAB with water.
13. Counterstain with hematoxylin (Innovex Product number NB305).
14. Quickly Rinse hematoxylin with tap water by filling and emptying the slide holder vessel with tap water until water runs clear.
15. Mount slides with xylene based mounting media OR mount from water with permanent "Advantage Mounting Media" (Innovex Product number NB300).

Substrate/Chromogen Mixing Protocol

Mix DAB by adding 1 drop of DAB chromogen (component 2) to 1 ml of Substrate Buffer (component 1) in the provided graduated mixing tube and mix. Left over mixed DAB substrate/chromogen solution is stable for 1-week when kept refrigerated and can be re-used. This minimizes DAB waste and disposal costs.

When darker DAB stain is desired, apply Innovex Quick DAB Enhancer solution (product number NB308) for 3-minutes at room temperature following the water rinse after DAB development. This DAB enhancer does not cause background.

STORAGE CONDITIONS

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

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

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