

## IMMUNOHISTOPROBE

### Two Step Polymer

### Alkaline Phosphatase Polymer Conjugated

### Goat anti-Mouse IgG (H+L) specific

### (min. cross to Hm, Rbt)

**Description:**

Two-Step Polymer Immunohistoprobe Plus is an extremely sensitive immunohistochemistry detection system. It is based on signal amplification by using a two-step polymer detection method. The primary antibody specific to an antigen on the tissue section is detected by an amplifier for primary antibody then followed by polymer detector. The antigen sites are then intensely visualized with an appropriate substrate/chromogen. Immunohistoprobe Plus does not use avidin or biotin, which completely eliminated nonspecific staining from endogenous avidin-biotin activity. It is currently the most sensitive detection system for IHC application.

**Cat No.:** 1821-08

**Lot No.**

**Volume:** Refer to the bottle label

**Reagent Provided:**

Reagent 1: Amplifier for Mouse IgG

Reagent 2: AP Polymer Detector

**Format:**

Reagent 1: Liquid with light yellow color

Reagent 2: Liquid with light red color

**Concentration:** Ready to use

**Application:** Immunohistochemistry for frozen and paraffin embedded tissues.

**Intended Usage:**

Immunohistoprobe kit is intended for use with primary antibodies from animals (mouse, rabbit, goat or rat) for localization and identification of specific antigens on a slide mounted tissue sections by light microscopy. Refer to section of "**Specificity and cross reactivity**".

**Host species:** Goat

**Specificity:** Mouse IgG (H+L)

**Cross Reactivity:** minimal cross reactivity with human and rabbit serum proteins.

**Preservative:** Proclin 300

**Conjugated enzyme:** Polymerized Alkaline Phosphatase

**Storage:** Store at 2-8°C. Stable up to 24 months (*see expiration date on bottle*). Do not mix the reagents from different lot.

**Expiration Date:** Refer to the bottle label

**Disclaimer:** For In vitro Laboratory Use Only. Not for diagnostic or therapeutic use.

**Suggested Protocol:**

***Users must be trained in immunohistochemical technique prior to undertaking the following protocol:***

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. De-paraffinize sections in xylene or xylene substitutes.
3. Re-hydrate through graded alcohols. Wash slides in distilled H<sub>2</sub>O.
4. Block Endogenous Alkaline Phosphatase Activity (optional, but necessary when endogenous alkaline phosphatase activities are concerned).
5. Perform antigen retrieval as required (see Recommendations for Use for primary antibody). Wash in wash buffer for 3 x 3 minutes.
6. Incubate with optimally diluted primary antibody (see Recommendations for Use for primary antibody). Wash in wash buffer for 3 x 3 minutes.
7. Incubate with **Reagent 1 (Amplifier for Mouse)**, ready to use for 10-15 minutes. Wash in wash buffer for 3 x 3 minutes.
8. Incubate with **Reagent 2 (AP Polymer Detector)**, ready to use for 10-15 minutes. Wash in wash buffer for 3 x 3 minutes.
9. Add chromogen working solution until color development finish. Rinse slides in distilled H<sub>2</sub>O.
10. Counterstain, and mount in appropriate mounting medium.

**REFERENCES:**

1. Bisgaard K, Pluzek KP: Use of polymer conjugates in immunohistochemistry: A comparative study of a traditional staining method to a Staining method utilizing polymer conjugates. Abstract XXI Intl Cong Intl Acad Pathol and 12<sup>th</sup> World Cong Acad Environ Pathol. Budapest, Hungary, October 20-25, 1996.
2. Shi ZR, Itzkowitz SH, Kim YS: A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcinoma Tissues J Histochem Cytochem 36:317-322, 1988.
3. Shi ZR, Au A, Soriano R et al: Non-Biotin Amplification (NBA) kit prevents nonspecific background staining of endogenous biotin induced by Heat Induced Epitope Retrieval (HIER) procedure. The J Histotechnol 23:327, 2000.
4. Shi SR, Key ME, Kalra KL: Antigen retrieval in formalin-fixed paraffin embedded tissues: An enhanced method for immunohisto-chemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39:741-748, 1991.
5. Shan-Rong Shi, James Guo, Richard J. Cote, Lillian Young, Debra Hawes, Yan Shi, Sandra Thu and Clive R. Taylor, Applied Immunohistochemistry & Molecular Morphology, vol 7, 201-208, 1999.