

HRP- Enhancing Wash Buffer For Peroxidase (HRP) Staining Systems

Technical Data Sheet

Reagent Category

Signal Amplification Wash Buffer

For immuno-peroxidase enzyme immunoassays

Specific Reagents Supplied

- 4 liters of HRP-Enhancing Wash Buffer
- 1 liter of HRP-Enhancing Wash Buffer

Product # NB301 (4 liters), NB301S (1 liter)

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#### **INTRODUCTION**

Horseshradish peroxidase (HRP) enzyme is often utilized as the labeling tag in a variety of immunoassays. Peroxidase enzyme is often conjugated to a variety of molecules such as antibodies, avidin, streptavidin, dextrin for performing immunohisto/cytochemical and ELISA procedures. When HRP enzyme is incubated with its appropriate substrate and a chromogenic substance, it results in color development. The color development registers the enzyme concentration by qualitative and/or quantitative means, which in essence determines the degree of the presence of the target antigen in the specimen tested by the respective assays.

#### **PRODUCT DESCRIPTION**

**HRP-Enhancing Wash Buffer** is a proven formulation developed for signal amplification of enzyme immunochemical assays utilizing horseradish peroxidase as the labeling enzyme. The use of HRP-Enhancing Wash Buffer for the rinsing steps of enzyme immunoassays greatly enhances the staining results for assays that employ horseradish peroxidase enzyme. This reagent is especially useful in the rinsing steps of tissue sections or cytosmear preparations in immunoperoxidase IHC and ICC staining procedures where the results are examined morphologically. The use of **HRP-Enhancing Wash Buffer** in place of phosphate buffered saline (PBS), tris buffer results in enhanced, bright and clearly resolved stains that are easier and more distinct to view. Its use allows the user to **reduce or eliminate enzyme digestion. HRP-Enhancing Wash Buffer can allow for increase in dilution of the primary antibodies or shorter incubation times for the primary antibodies.** In addition, rinsing with **HRP-Enhancing Wash Buffer** allows for shortening and standardizing the chromogen incubation step which can be unpredictable and vary with primary antibodies and tissues. The use of this enhancing rinsing buffer further enhances the quality of chromogen staining and allows the chromogen color to develop much faster. **HRP-Enhancing Wash Buffer** amplifies weak staining and minimizes false negative results.

#### **APPLICATION / INTENDED USE**

**HRP-Enhancing Wash Buffer** is intended for the rinsing steps in immunoperoxidase staining procedures, HRP-Enhancing Wash Buffer can be used in automated stainers.

#### **STORAGE CONDITIONS**

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

#### **PRODUCT FORMAT**

Working solution; No dilution or adjustments necessary.

**CONTINUED NEXT PAGE**

## INSTRUCTIONS

### Specimen Preparation for Immuno Histo/Cytochemical Staining

*Prepare sections as usual, the following is a general guideline.*

**For paraffin sections:** deparaffinize sections and rehydrate in water; Perform peroxidase blocking and retrieval and proceed to step 1 below.

**For frozen sections:** cut sections, dry, and fix in cold acetone or the fixative of choice. Incubate in PBS for 3- minutes at room temperature. Use HRP-Enhancing Wash Buffer in place of PBS or tris-Buffered Saline for the rinse steps as described below.

**For cytocentrifuge preparations:** prepare cytocentrifuge preparations of cell suspensions and proceed with immunoperoxidase staining as usual. Use HRP-Enhancing Wash Buffer in place of PBS or tris-Buffered Saline for the rinse steps as described below:

1. Apply primary antibody and incubate according to manufacturer's recommended incubation time.
2. **Rinse with HRP-Enhancing Wash Buffer 2-times** for 5 seconds each time for Innovex detection kits, follow manufacturer's recommended rinsing time for detection kits employed.
3. Apply the secondary linking antibody or pre-polymer solution and incubate according to manufacturer's instruction.
4. **Rinse with HRP-Enhancing Wash Buffer 2-times** for 5 seconds each for Innovex detection kits, follow manufacturer's recommended rinsing time for detection kits employed.
5. Apply HRP-enzyme label or Polymer solution and incubate according to manufacturer's instruction.
6. **Rinse with HRP-Enhancing Wash Buffer 2-times** for 5-seconds each for Innovex detection kits, follow manufacturer's recommended rinsing time for detection kits employed.
7. **Rinse with HRP-Enhancing Wash Buffer 2-times** for 5 seconds each for Innovex detection kits follow manufacturer's recommended rinsing time for detection kits employed.
8. Apply substrate-chromogen (AEC or DAB) and note that chromogens will develop quicker when using the HRP-Enhancing Wash Buffer as the rinse buffer. Therefore, when employing HRP-Enhancing Wash Buffer for the first time, develop chromogen under the microscope and time the color development for the particular chromogen employed; Note this incubation time for future work.
9. Rinse in DI water.
10. Counterstain with aqueous based hematoxylin (Innovex Product # NB305) and mount with aqueous based permanent mounting media (Innovex, Advantage mounting media, Product #NB300)

**\* Do not leave slide(s) in Enhancing Wash Buffer for more than 3-minutes. If an extended pause needs to take place, store slide(s) in PBS for duration of pause.**

### For ELISA Assays

HRP-Enhancing Wash Buffer can be used equally as well for the washing of ELISA plates; however, some Triton should be added to the solution. Add 0.25 ml Triton X-100™ (Sigma Chemicals, St. Louis, MO) to 500 ml of HRP-Enhancing Wash Buffer.

**FOR PROFESSIONAL AND RESEARCH USE**

**FOR ADDITIONAL TECHNICAL SUPPORT**

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