

HRP- Enhancing Wash Buffer 5X Concentrate

For IMMUNO-PEROXIDASE (IHC) STAINING

Technical Data Sheet

Reagent Category

Signal Amplification Wash Buffer

For immuno-peroxidase Staining

Product # NB301-5C (5X Concentrate)

Specific Reagents Supplied

☐ 1 Liter of 5X Enhancing Wash Buffer

INTRODUCTION

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Horseradish 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 and streptavidin for performing immunohistochemical 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 test specimens

### PRODUCT DESCRIPTION

**HRP-Enhancing Wash Buffer** is a proven formulation 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 HRP enzyme immunoassays greatly enhances the staining results. This reagent is especially useful in the rinsing steps of tissue sections or cytosmear preparations in immunoperoxidase-IHC staining procedures where the results are examined morphologically. The use of **HRP-Enhancing Wash Buffer** in place of PBS, Tris or other wash buffers for the rinsing steps results in enhanced, bright and clearly resolved stains that is easier and more distinct to view. Its use allows the user to **reduce or eliminate enzyme digestion or heat retrieval application pre-steps for some primary antibodies**. Furthermore, the HRP-Enhancing Wash Buffer can allow for **increased dilution of the primary antibody and/or shorter incubation time of the primary antibody**. In addition the use of **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** also greatly assists with minimizing false negative results.

### APPLICATION / INTENDED USE

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

### PRODUCT FORMAT

5X Concentrate; Dilute 1:5 prior to use (1 volume of 5X concentrate of Enhancing Wash Buffer and 4 volume of Deionized Water). Mix well.

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## STORAGE CONDITIONS

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

## 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. **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. **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 as described below:

1. Rinse slides for 10 -seconds in HRP-Enhancing Wash Buffer prior to the application of primary antibody.
2. Apply primary antibody and incubate according to manufacturer's recommended incubation time.
3. Rinse with HRP-Enhancing Wash Buffer for 10-seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacturer's recommended time for rinse steps.
4. Apply Secondary Linking antibody OR HRP-Polymer and incubate according to manufacturer's instruction. IF USING HRP-POLYMER, SKIP STEPS 6 and 7 and CONTINUE WITH STEP 8.
5. Rinse with HRP-Enhancing Wash Buffer for 10-seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacturer's recommended time for rinse steps.
6. Apply HRP-enzyme label and incubate according to manufacturer instruction.
7. Rinse with HRP-Enhancing Wash Buffer for 10 seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacturer's recommended time for rinse steps.
8. Apply substrate-chromogen (AEC or DAB) and note that chromogens will develop much quicker when using the HRP-Enhancing Wash Buffer as the rinse buffer.
10. Counterstain with aqueous based hematoxylin (Innovex Product # NB305) and mount with aqueous based permanent mounting media (Innovex, Advantage mounting media, Product #NB300) OR with organic based mounting media.

If using AEC chromogen, avoid mounting with organic solvent based mounting media.

**\* Do not leave slide(s) in Enhancing Wash Buffer for more than 1 minute. If an extended pause needs to take place, store slide(s) in PBS.**

### **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.

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**FOR ADDITIONAL TECHNICAL SUPPORT**  
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