INNOVEX BIOSCIENCES

Cyto Q
HRP- Enhancing Wash Buffer
For Horseradish Peroxidase Staining System

Technical Data Sheet

Reagent Category
Signal Amplification Wash Buffer
for immuno-peroxidase enzyme immunoassays

Antibody Formats Supplied
- **4 liters of HRP-Enhancing Wash Buffer**
- **1 liter of HRP-Enhancing Wash Buffer**

Item#: IMI00392E (Liter) or IMI00396E (4 Liter)

INTRODUCTION
Horseradish peroxidase (HRP) enzyme is often utilized as the labeling tag in a variety of immunoassay types. Peroxidase enzyme is often conjugated to a variety of molecules such as antibodies, avidin and strepavidin for performing immunohisto/cytochemical and ELISA procedures. When HRP enzyme is incubated for a short time with its appropriate substrate and a chromogenic substance, it results in color development. The rate of color development measures 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 newly formulated reagent 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 that employ horseradish peroxidase enzyme label greatly enhances the staining results. This reagent is especially useful in rinsing steps of tissue sections or cytosmear preparations in immunoperoxidase staining procedures where the results are examined morphologically.

The use of **HRP- Enhancing Wash Buffer** in place of phosphate buffered saline (PBS) or other commonly used wash buffers for rinsing steps of immunoperoxidase staining procedure results in enhanced, bright and clearly resolved stains that are easier and more distinct to view. Its use further allows the user to **eliminate enzyme digestion or heat application pre-steps in majority of the cases, to increase the dilution of primary antibodies and /or shorten the employed incubation time of the primary antibody incubation time**. In addition the use of **HRP- Enhancing Wash Buffer** allows for shortening and standardizing chromogen incubation step which is usually long and unpredictable and it varies 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 procedure. HRP-Enhancing Wash Buffer can be used in automated stainers.

CONTINUED OVER

PRODUCT FORMAT

Working solution, **no** dilution or adjustments necessary.

STORAGE CONDITIONS

Store in refrigerator at 2-8ºC until the expiration date noted on the container.

INSTRUCTIONS

Specimen Preparation for Immuno/Histochemical 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, simply replace HRP-Enhancing Wash Buffer in place of PBS or Tris-buffered saline as described below:

1. Rinse slides for 30 seconds to 1 minute in HRP-Enhancing Wash Buffer prior to the application on primary antibody.
2. Apply primary antibody and incubate for **only half the usually employed incubation time or increase dilution of primary by 2-4 folds.**
3. **Rinse in HRP-Enhancing Wash Buffer 2X** for 5 seconds each. For detection kits other then Innovex rinse 2x for 30 seconds each.*
4. Apply the secondary multivalent linking antibody and incubate according to manufacturer's instruction.
5. Rinse in **HRP-Enhancing Wash Buffer 2x** for 5 seconds each. For detection kits other then Innovex rinse 2x for 30 seconds each.*
6. Apply HRP-enzyme label and incubate according to manufacturer instruction.
7. Rinse in **HRP-Enhancing Wash Buffer 2x** for 5 seconds each. For detection kits other then Innovex rinse 2x for 30 seconds each.*
8. Apply substrate-chromogen (AEC or DAB) and note that chromogens will develop much quicker when using the HRP-Enhancing Wash Buffer as the rinsing buffer. Therefore, when employing "HRP-Enhancing buffer" for the first time, develop chromogen under the microscope and time the color development for the particular chromogen used. Note this incubation time for future work.
9. Rinse in Dl water.
10. Counterstain with aqueous based and mount with aqueous based permanent 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-10™ (Sigma Chemicals, St. Louis, MO) to 500 ml of HRP-Enhancing Wash Buffer.

FOR IN VITRO RESEARCH USE ONLY

FOR ADDITIONAL TECHNICAL SUPPORT:

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