

Alkaline Phosphatase-Enhancing Wash Buffer 5X Concentrate

For Alkaline Phosphatase Staining Systems

Technical Data Sheet

Reagent Category

Signal Amplification Wash Buffer
For immuno-alkaline phosphatase

Specific Reagents Supplied

□ 1 liter of 5X Alkaline Phosphatase Enhancing Wash Buffer

Product # NB302-5C (1 liter)

INTRODUCTION

Alkaline phosphatase enzyme is often utilized as the labeling tag in a variety of immunoassay types. Alkaline phosphatase enzyme is often conjugated to a variety of molecules such as antibodies, avidin and streptavidin for performing immunohistochemical and ELISA procedures. When Alkaline phosphatase 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

Innovex Alkaline Phosphatase-Enhancing Wash Buffer is a proven formulation for signal amplification of enzyme immunochemical assays employing alkaline phosphatase enzyme as the labeling enzyme. The use of **Alkaline Phosphatase-Enhancing Wash Buffer** for the rinsing steps of enzyme immunoassays enhances the staining results. This solution is especially useful in the rinsing steps of tissue sections or cytosmear preparations in immuno-alkaline phosphatase staining procedures.

The use of **Alkaline Phosphatase-Enhancing Wash Buffer** in place of PBS or Tris or other commonly used wash buffers for the rinsing steps 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 or heat retrieval application pre-steps. Furthermore, the Alkaline Phosphatase Enhancing Wash Buffer can allow for an increase dilution of the primary antibody and/or shorter incubation time of the primary antibody.**

In addition, the use of **Alkaline Phosphatase-Enhancing Wash Buffer** allows for shortening and standardizing the chromogen incubation step which can be unpredictable and it 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 quicker, therefore, shortening the chromogen incubation step. **Alkaline Phosphatase-Enhancing Wash Buffer** greatly assists with minimizing false negative results.

APPLICATION / INTENDED USE

Alkaline Phosphatase-Enhancing Wash Buffer is intended for the rinsing steps involved in immuno-alkaline phosphatase staining procedures. **Alkaline Phosphatase-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 Alkaline Phosphatase Enhancing Wash Buffer and 4 volume of Deionized Water). Mix well.

STORAGE CONDITIONS

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

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INSTRUCTIONS

Specimen Preparation for Immuno histochemical Staining

Prepare section 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. **For cytocentrifuge preparations:** prepare cytocentrifuge preparations of cell suspensions and proceed with immuno-alkaline phosphatase staining as usual. Use Alkaline Phosphatase-Wash Buffer in place of PBS or Tris Buffered Saline as instructed below:

1. Rinse slides for 10 seconds in **Alkaline Phosphatase-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 Alkaline Phosphatase-Enhancing Wash Buffer for 10 seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacture's recommended time for rinse steps.
4. Apply secondary linking antibody and incubate according to manufacturer's instruction.
5. Rinse with Alkaline Phosphatase-Enhancing Wash Buffer for 10 seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacture's recommended time for rinse steps.
6. Apply alkaline phosphatase label and incubate according to manufacturer instruction.
7. Rinse with Alkaline Phosphatase-Enhancing Wash Buffer for 10 seconds for Innovex staining kits. For other staining kits and autostainers follow the manufacture's recommended time for rinse steps.
8. Apply appropriate substrate-chromogen, e.g., Fast Red or New Fuchsin and note that chromogens will develop much quicker when using the **Alkaline Phosphatase-Enhancing Wash Buffer** as the rinsing buffer. Therefore, when employing **Alkaline Phosphatase-Enhancing Wash Buffer** for the first time, view the specimen under the microscope with the chromogen on the slide and time the color development for desired intensity for the particular chromogen used note this incubation time for future work.
9. Rinse with 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)

***If an extended pause needs to take place, slide(s) can be stored in Alkaline Phosphatase-Enhancing Wash Buffer.**

For ELISA Assays

Alkaline Phosphatase-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 Alkaline Phosphatase-Enhancing Wash Buffer.

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