

Cyto Q

Alkaline Phosphatase-Enhancing Wash Buffer

For Alkaline Phosphatase Staining Systems

Technical Data Sheet

Reagent Category

Signal Amplification Wash Buffer

For immuno-alkaline phosphatase staining

Specific Reagents Supplied

4 liters of working solution

1 liter of working solution

Product # NB302 (4 liters), NB302S (1 liter)

INTRODUCTION

Alkaline phosphatase enzyme is often utilized as the labeling tag in a variety of immunoassay types. This enzyme is often conjugated to a variety of molecules such as antibodies, avidin and streptavidin for performing immunohisto/cytochemical and ELISA assays. 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 specimen tested by the respective assays.

PRODUCT DESCRIPTION

Innovex Alkaline Phosphatase-Enhancing Wash Buffer is a proven formulation developed 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 that employ alkaline phosphatase enzyme label. This reagent 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 phosphate buffered saline (PBS) or other commonly used wash buffers for the rinsing steps of alkaline phosphatase staining procedures 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 application pre-steps in the majority of cases. Furthermore, the Alkaline Phosphatase Enhancing Wash Buffer can allow for an increase dilution of the primary antibody and/or shorten the 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 may vary with primary antibodies and tissues employed. 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

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

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; Perform 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 Alkaline Phosphatase-Enhancing Wash Buffer 1-minute at room temperature prior to application of primary antibody.

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 30-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 slides with Alkaline Phosphatase-Enhancing Wash Buffer 2-times for 1-3 minutes each time.
4. Apply Alkaline phosphatase-Polymer or the biotinylated Secondary Linking antibody and incubate according to manufacturer's instruction.
5. Rinse in Alkaline Phosphatase-Enhancing Wash Buffer 2-times for 1-3 minutes each time.
6. Apply alkaline phosphatase-streptavidin when using Biotin -streptavidin system and incubate according to manufacturer instruction.
7. Rinse in Alkaline Phosphatase-Enhancing Wash Buffer 2 times for 1- 3 minutes each time.
8. Apply appropriate substrate-chromogen, e.g., Fast Red or New Fuchsin, permanent red, etc.; Note that chromogens will develop much quicker when using the **Alkaline Phosphatase-Enhancing Wash Buffer** as the rinse 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 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)

***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, for ELISA assays 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.

FOR PROFESSIONAL AND RESEARCH USE

FOR ADDITIONAL TECHNICAL SUPPORT

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