



Cyto Q

Alkaline Phosphatase-Enhancing Wash Buffer For use with alkaline Peroxidase staining system

Technical Data Sheet

Reagent Category

Signal Amplification Wash Buffer
For immuno-alkaline phosphatase
enzyme immunoassays

Antibody Formats Supplied

- 4 liters of working solution
- 1 liter of working solution

PRODUCT# IMI00592E (Liter) | IMI00596E (4 Liters)

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 this 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

Innovex "Alkaline phosphatase-Enhancing Wash Buffer" is a newly formulated reagent 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 that employ alkaline phosphatase enzyme label greatly enhances the staining results. Enhancing wash buffer is especially useful in rinsing steps of tissue sections or cytosmear preparations in immuno-alkaline phosphatase staining procedures where the results are examined morphologically.

The use of "Alkaline phosphatase-Enhancing Wash Buffer" in place of commonly used buffers such as phosphate buffered saline (PBS) or similar wash buffers gives rise to bright and clearly resolved staining patterns that are easy and distinct to view. In addition its use enables 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 "Alkaline phosphatase-Enhancing wash buffer" further 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 quicker, therefore, shortening the chromogen incubation step. **"Alkaline phosphatase enhancing wash buffer" greatly assists with minimizing false negative results.**

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INTENDED USE/APPLICATION

“Alkaline phosphatase-Enhancing wash buffer” is intended for the rinsing steps involved in immuno-alkaline phosphatase staining procedure.

PRODUCT FORMAT

Working solution, no dilution or adjustments necessary.

STORAGE CONDITIONS

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

INSTRUCTIONS

Specimen Preparation for Immunocytochemical 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 “Alkaline phosphatase-Enhancing Wash Buffer” 1 minute at room temperature.
 - **For cytocentrifuge preparations:** Prepare cytocentrifuge preparations of cell suspensions and proceed with immuno-alkaline phosphatase staining as usual, simply replace “Alkaline phosphatase-Wash Buffer” in place of PBS or Tris-buffered saline as instructed below:
1. Immerse slides into a container containing “Alkaline phosphatase-wash buffer” prior to the application of 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 “Alkaline phosphatase-wash buffer” twice.
 4. Apply the multivalent linking antibody and incubate according to manufacturer’s instruction.
 5. Rinse in “Alkaline phosphatase-wash buffer” twice.
 6. Apply alkaline phosphatase label and incubate according to manufacturer instruction.
 7. Rinse in “Alkaline phosphatase-wash buffer ” twice.
 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-wash buffer” as the rinsing buffer. Therefore, when employing “Alkaline phosphatase-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 in water.
 10. Counterstain with aqueous based hematoxylin and mount with aqueous based permanent mounting media.

For ELISA Assays

“Alkaline phosphatase-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-100TM (Sigma Chemicals, St. Louis, MO) to 500 ml of alkaline phosphatase-Wash Buffer.