



Instructions For Use AET080-IFU

Rev. Date: 10/24/03

Revision: 4

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P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

UltraTek Alk-Phos Anti-Mouse (Fast Red) Staining System

Description: 70 Slide Kit.

Species of Origin: Goat

Antigen Specificity: Anti-Mouse IgG(H+L)

Preadsorbed Against: Human, Bovine, Horse, Rabbit, Swine

Enzyme Conjugate: Alkaline Phosphatase

Chromogen Substrate: Fast- Red

Uses/Limitation: Do not use past expiration date.

For immunohistochemical studies.

Contents:

 Item #
 Volume

 Super Block
 8 ml

 Anti-Mouse
 8 ml

 Alk-Phos
 8 ml

 Fast-Red Tablets
 8 Tablets

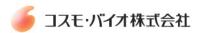
 Naphthol Phosphate Buffer
 8x5 ml

Storage: 2-8° Centigrade.

Procedure:

- Deparaffinize and rehydrate tissue section.
- Wash 2 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- If required, incubate tissue in digestive enzyme (catalog # PSS060 or TSS155) or Citrate Plus (catalog # CPL500).
- 4. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 5. Apply Super Block (blue cap), and incubate for 5 minutes at room temperature to block nonspecific background staining. Note: Do not exceed 10 minutes.
- Wash 1 time in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 7. Apply primary antibody and incubate according to manufacturer's protocol.
- 8. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- Apply UltraTek Anti-Mouse (yellow cap), and incubate for 10 minutes at room temperature.
- 10. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 11. Apply UltraTek Alk-Phos (red cap), and incubate for 10 minutes at room temperature.

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- 12. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 13. Add 1 Fast-Red tablet to a vial of Naphthol Phosphate Buffer and shake until tablet is dissolved. Apply to tissue section for 10-20 minutes or until desired stain intensity is achieved.
- 14. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 15. For optimal results counterstain using Hematoxylin for Automation (catalog # HAQ500).
- 16. Coverslip using Aqueous mounting media (catalog # AMT030 or PMT030).

Precautions: Handle with care and dispose of according to all regulations.

Troulbleshooting Guide

Overstaining:

- Concentration of the primary antibody was too high or the incubation time was too long.
- 2. Temperature during incubation was too high.
- 3. Incubation time with link antibody or streptavidin/enzyme label was too long.

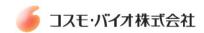
Nonspecific Background Staining:

- 1. Rinsing between steps was inadequate.
- 2. Tissue was allowed to dry with reagents on.
- Folds in tissue trapped reagents.
- 4. Tissue contains endogenous alkaline phosphatase.
- 5. Tissue contains endogenous biotin.
- 6. Antigen migrated in tissue.
- 7. Excessive tissue adhesive on slides.
- 8. Inadequate blocking with protein block.

Weak Staining:

- Primary antibody concentration was too low or incubation time was too short.
- 2. Reagents are past their expiration date.
- 3. Inadequate removal of wash water between steps, resulting in dilution of reagents.
- 4. Counterstain or mounting media were incompatible and dissolved the chromogen reaction product.
- Room temperature was excessively cool.

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- 6. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
- 7. Excessive incubation with protein block (Super Block).

No Staining:

- 1. Steps were inadvertently left out.
- 2. There is no antigen in the tissue.
- 3. The primary antibody is not of mouse origin.
- 4. Chromogenic substrate has been replaced with another that is not intended for use with alkaline phosphatase.