ScyTek Laboratories

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: 2-8º Centigrade.

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
3. 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.
6. 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).
9. 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:

1. 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.
3. 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:

1. 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.
5. 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.