P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

EconoTek Alk-Phos Anti-Polyvalent (Fast-Red) Stain Kit

Species of Origin: Goat
Antigen Specificity: Anti-Mouse, Rat, Rabbit, Guinea Pig
Preadsorbed Against: Human
Enzyme Conjugate: Alkaline Phosphatase
Chromogen Substrate: Fast Red

Components:

Description	Volume
Super Block	8 ml
EconoTek Anti-Polyvalent	8 ml
EconoTek Alk-Phos	8 ml
Fast Red Tablets	8 Tablets
Naphthol Phosphate Buffer	5 ml x 8 vials

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. If required, incubate tissue in digestive enzyme.
3. Wash 4 times in buffer.
4. Apply Super Block, and incubate for 5-10 minutes at room temperature to block nonspecific background staining. Note: Do not exceed 10 minutes or there may be a reduction in desired stain.
5. Wash 1 time in buffer.
6. Apply primary antibody and incubate according to manufacturer's protocol.
7. Wash 4 times in buffer.
8. Apply EconoTek Anti-Polyvalent (Yellow Solution), and incubate for 30 minutes at room temperature.
9. Wash 4 times in buffer.
10. Apply EconoTek Alk-Phos (Red Solution), and incubate for 30 minutes at room temperature.
11. Rinse 2 times in buffer.
12. Add 1 Fast Red Tablet to 1 vial of Naphthol Phosphate Buffer and shake until tablet is fully dissolved.
13. Rinse slide 1 time in distilled water.
14. Apply Fast Red Solution to tissue and incubate for 15 minutes.
15. Rinse 2 times in distilled water.
16. Counterstain and coverslip.

Storage: 2° C 8° C

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- Troubleshooting 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.
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, rat, rabbit or guinea pig origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with alkaline phosphatase.