

## Instructions For Use AEY080-IFU

Rev. Date: May 27, 2011

**Revision: 1** 

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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

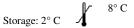
Chromogen Substrate: Fast Red

Components:Description<br/>Super BlockVolume<br/>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.
- 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.









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## - Troubleshooting 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.
- 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.
- 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:

- 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.

