ScyTek<br/>LaboratoriesInstructions For Use<br/>MTM004-IFURev. Date: 10/28/03Revision: 2Page 1 of 2

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# Mouse To Mouse Alk-Phos Ready-To-Use

Species of Origin: Antigen Specificity: Preadsorbed Against: Enzyme Conjugate: Chromogen Substrate:

Goat Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig) Human Alkaline Phosphatase Fast- Red

#### **Procedure:**

- 1. Deparaffinize and rehydrate tissue section.
- 2. Wash 2 times in buffer.
- 3. If required, incubate tissue in digestive enzyme.
- 4. Wash 4 times in buffer.
- 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 or there may be a reduction in desired stain.
- 6. Wash 1 time in buffer.
- 7. Apply Mouse to Mouse Block and incubate10-60 minutes. Incubation time is dependent on the amount of endogenous Ig found in the tissue.
- 8. Wash 4 times in buffer.
- 9. Apply primary antibody and incubate according to manufacturer's protocol.
- 10. Wash 4 times in buffer.
- 11. Apply UltraTek Anti-Polyvalent (yellow cap), and incubate for 10 minutes at room temperature.
- 12. Wash 4 times in buffer.
- 13. Apply UltraTek Alk-Phos (red cap), and incubate for 10 minutes at room temperature. 14.Rinse 4 times in buffer.
- 15. 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. **WARNING:** Handle with care and dispose of according to all regulations.
- 16. Counterstain and coverslip using an aqueous mounting media.

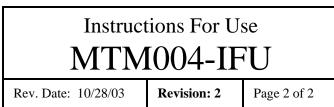
# **Troubleshooting Guide**

# Overstaining:

1. Concentration of the primary antibody was too high or the incubation time was too long.

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- 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.