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Mouse To Mouse Alk-Phos Ready-To-Use (Large Volume)

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

Goat Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig) Alkaline Phosphatase None

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 (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.
- 5. Wash 1 time in buffer.
- 6. Apply Mouse To Mouse Block and incubate 10-60 minutes. Incubation time is dependent on the amount of endogenous Ig found in the tissue type.
- 7. Rinse 4 times in buffer.
- 8. Apply primary antibody and incubate according to manufacturer's protocol.
- 9. Wash 4 times in buffer.
- 10. Apply UltraTek Anti-Polyvalent (yellow cap), and incubate for 10-20 minutes at room temperature.
- 11. Wash 4 times in buffer.
- 12. Apply UltraTek Alk-Phos (red cap), and incubate for 10-20 minutes at room temperature.
- 16. Rinse 4 times in buffer.
- 17. Apply chromogen intended for use with Alkaline Phosphatase.
- 18. Counterstain and coverslip.

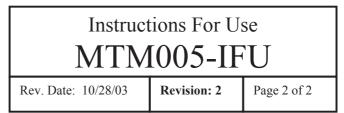
Troubleshooting Guide

Overstaining:

- 1. Concentration of the primary antibody was too high or the incubation time was too long.
- 2. Temperature during incubation was too high.

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3. Incubation time with UltraTek Anti-Polyvalent or UltraTek Alk-Phos 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. Inadequate blocking with Mouse To Mouse Block.
- 5. Tissue contains endogenous biotin.
- 6. Antigen migrated in tissue.
- 7. Excessive tissue adhesive on slides.
- 8. Inadequate blocking with Super 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.
- 7. Excessive incubation with 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.
- 5. One or more components of the kit have been inactivated.

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