ScyTek Laboratories

Instructions For Use MTM004-IFU		
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Mouse To Mouse Alk-Phos Ready-To-Use

Species of Origin: Goat
Antigen Specificity: Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig)
Preadsorbed Against: Human
Enzyme Conjugate: Alkaline Phosphatase
Chromogen Substrate: 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.