ScyTek Laboratories

Instructions For Use
MTM001-IFU

Rev. Date: 10/28/03	**Revision: 2**	Page 1 of 2

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Mouse To Mouse HRP Ready-To-Use

Species of Origin: Goat
Antigen Specificity: Anti-Polyvalent (Mouse, Rat, Rabbit and Guinea Pig)
Enzyme Conjugate: Peroxidase
Chromogen Substrate: Diaminobenzidine (DAB)
Description:

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
3. Wash 2 times in buffer.
4. If required, incubate tissue in digestive enzyme.
5. Wash 4 times in buffer.
6. 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.
7. Wash 1 time in buffer.
8. 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.
9. Rinse 4 times in buffer.
10. Apply primary antibody and incubate according to manufacturer's protocol.
12. Wash 4 times in buffer.
13. Apply UltraTek Anti-Polyvalent (yellow cap), and incubate for 10-20 minutes at room temperature.
14. Wash 4 times in buffer.
15. Apply UltraTek HRP (red cap), and incubate for 10-20 minutes at room temperature.
16. Rinse 4 times in buffer.
17. Add 4 drops (200ul) DAB Chromogen to DAB Substrate, mix by swirling and apply to tissue. Incubate for 5-15 minutes, depending on the desired stain intensity.
18. Counterstain and coverslip using a permanent mounting media.

Troubleshooting Guide

Overstaining:

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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 UltraTek Anti-Polyvalent or UltraTek HRP 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 peroxidase.
5. One or more components of the kit have been inactivated.