

ScyTek Laboratories

Instructions For Use AKA080-IFU

Rev. Date: 10/24/03

Revision: 3

Page 1 of 2

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Fax (435) 755-0015 - www.scytek.com

UltraTek HRP (Anti-Mouse) Ready-To-Use (70 slide)

Species of Origin: Goat

Antigen Specificity: Anti-Mouse IgG (H+L)

Preadsorbed Against: Human, Bovine, Horse, Rabbit, Swine

Enzyme Conjugate: Peroxidase

Chromogen Substrate: Diaminobenzidine (DAB)

Procedure:

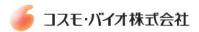
- 1. Deparaffinize and rehydrate tissue section.
- To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
- 3. Wash 2 times in buffer.
- 4. If required, incubate tissue in digestive enzyme.
- 5. Wash 4 times in buffer.
- 6. Apply 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 primary antibody and incubate according to manufacturer's protocol.
- 9. Wash 4 times in buffer.
- 10. Apply UltraTek Anti-Mouse (yellow cap), and incubate for 10 minutes at room temperature.
- 11. Wash 4 times in buffer.
- 12. Apply UltraTek HRP (red cap), and incubate for 10 minutes at room temperature.
- 13. Rinse 4 times in buffer.
- 14. 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. WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.
- 15. Counterstain and coverslip using a permanent mounting media.

Troubleshooting Guide

Overstaining:

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 peroxidase.
- Tissue contains endogenous biotin.
- 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.
- 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 or normal serum).

No Staining:

- 1. Steps were inadvertently left out.
- 2. There is no antigen in the tissue.
- 3. The primary antibody is not of mouse 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.