

## Instructions For Use ABJ-IFU

Rev. Date: 10/15/03

**Revision: 2** 

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

### UltraTek (Anti-Mouse) Ready-To-Use

**Description:** 

Species of Origin: Goat

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

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

Storage: Store at 2-8°C.

#### **Procedure:**

- Deparaffinize and rehydrate tissue section.
- (Optional) To reduce nonspecific background staining due to endogenous peroxidase (only when using peroxidase label), 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.
- (Optional) Place slide in Super Block, and incubate for 5-10 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. Rinse in buffer.
- 8. Apply primary antibody and incubate according to manufacturer's protocol.
- 9. Rinse in buffer.
- 10. Apply UltraTek (Anti-Mouse), and incubate for 10 minutes at room temperature.
- 11. Rinse in buffer.
- 12. Apply enzyme label, and incubate according to manufacturer's protocol.
- 13. Rinse in buffer.
- 14. Place slide in appropriate chromogenic substrate and incubate until desired reaction is achieved.
- 15. Counterstain and coverslip.



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#### **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.
- 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 enzyme.
- 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. Reagent is reaching the end of its useful life.
- 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 or normal serum).

#### No Staining:

- 1. Steps were inadvertently left out.
- 2. There is no relevant antigen in the tissue.

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- 3. The primary antibody is not of mouse origin.
- 4. Chromogenic substrate does not match enzyme label.
- 5. One or more components have been inactivated.