

Instructions For Use ABK-IFU

Rev. Date: 10/15/03

Revision: 2

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UltraTek (Anti-Rabbit) Ready-To-Use

Description:

Species of Origin: Goat

Antigen Specificity: Anti-Rabbit IgG (H+L) Preadsorbed Against: Human, Mouse, Rat.

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.
- 6. (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-Rabbit), and incubate for 10 minutes at room temperature.
- 11. Rinse in buffer.
- 12. Place slide in enzyme label, and incubate according to manufactures protocol.
- 13. Rinse in buffer.
- 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 it's 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.
- 3. The primary antibody is not of rabbit origin.

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- 4. Chromogenic substrate does not match enzyme label.
- 5. One or more components have been inactivated.