ScyTek Laboratories

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

UltraTek (Anti-Polyvalent) Ready-To-Use

Description:

Species of Origin:	Goat
Antigen Specificity:	Anti-Mouse, Rat, Rabbit, Guinea Pig IgG (H+L)
Preadsorbed Against:	Human

Uses/Limitations: This reagent is provided in bulk form for automated systems or can be poured in staining jars and used repeatedly. If the DIP method is employed, staining jar should be sealed and refrigerated between uses. Remove staining jars from the refrigerator 30 minutes prior to staining to allow reagents to come to room temperature.

Storage: Store at 2-8°C.

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. (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 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-Polyvalent, and incubate for 10 minutes at room temperature.
11. Rinse in buffer.
12. Apply enzyme label, and incubate according to manufacturer’s protocol.

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13. Rinse in buffer.

14. Place slide in appropriate chromogenic substrate and incubate until desired reaction is achieved.

15. Counterstain and coverslip.

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:

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1. Steps were inadvertently left out.
2. There is no relevant antigen in the tissue.
3. The primary antibody is not of mouse, rat, rabbit or guinea pig origin.
4. Chromogenic substrate does not match enzyme label.
5. One or more components have been inactivated.