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

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

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

Immunohistochemical Staining Protocol:

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 primary antibody and incubate according to manufacturer's protocol.
9. Wash 4 times in buffer.
10. Apply UltraTek Anti-Polyvalent (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.

WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

14. Add 8 drops of DAB chromogen to one 5ml vial of DAB Substrate. Mix well and apply to tissue for 5 minutes.
15. Rinse 1 time in DI Water.

Storage: 2° C  8° C


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16. Apply DAB Chromogen/Substrate mixture and incubate for a second 5-minute period.

17. Rinse 3 times DI water.

18. Counterstain and coverslip using a permanent mounting media.

-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 Backgroung 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.
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. 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 in high enough amounts.

Storage: 2° C 8° C

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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, 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.