

Instructions For Use AKC080-IFU

Rev. Date: Nov. 5, 2021

Revision: 6

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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 HRP (Anti-Rabbit) Ready-To-Use (70 slide)

Species of Origin: Goat

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

Enzyme Conjugate: Peroxidase Chromogen Substrate: Diaminobenzidine

Procedure:

- 1. De-parafinize and re-hydrate tissue section.
- To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in Peroxide Block for 10-15 minutes.
- 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-Rabbit (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.

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









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- 17. Rinse 3 times DI water.
- 18. 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.

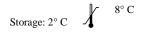
Nonspecific Background Staining:

- 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 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.
- 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).

No Staining:









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- 1. Steps were inadvertently left out.
- 2. There is no antigen in the tissue.
- 3. The primary antibody is not of rabbit origin.
- 4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.



