

Instructions For Use UHR-IFU

Rev. Date: Sept. 8, 2008

Revision: 1

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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 Lab Pack

Species of Origin:GoatAntigen Specificity:Anti-RabbitPreadsorbed Against:Human

Enzyme Conjugate: Horseradish Peroxidase

Chromogen Substrate: None Provided

Uses/Limitations: Do not use past expiration date.

For immunohistochemical studies.

Availability:

REF # Volume

UHP125 125ml Super Block, 125ml UltraTek Anti-Rabbit, 125ml UltraTek HRP.

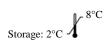
UHP500 500ml Super Block, 500ml UltraTek Anti-Rabbit, 500ml UltraTek HRP.

UHP999 1000ml Super Block, 1000ml UltraTek Anti-Rabbit, 1000ml UltraTek HRP.

Storage: 2-8° Centigrade.

Procedure:

- 1. Deparaffinize and rehydrate tissue section.
- Wash 2 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- If required, incubate tissue in digestive enzyme (catalog # PSS060 or TSS155) or Citrate Plus (catalog # CPL500).
- 4. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- Apply 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.
- 6. Wash 1 time in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 7. Apply primary antibody and incubate according to manufacturer's protocol.
- 8. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 9. Apply UltraTek Anti-Rabbit (yellow solution), and incubate for 10 minutes at room temperature.









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- 10. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 11. Apply UltraTek HRP (red solution), and incubate for 10 minutes at room temperature.
- 12. Wash 3 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
- 13. Apply chromogen intended for use with Horseradish Peroxidase and incubate as desired.
- 14. For optimal results counterstain using Hematoxylin for Automation (catalog # HAQ500).
- 15. Coverslip using mounting media of choice (catalog # AMT030 or PMT030).

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.
- Folds in tissue trapped reagents.
- 4. Tissue contains endogenous peroxidase.
- 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.



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- 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).

No Staining:

- 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 Horseradish Peroxidase.

Precautions: Handle with care and dispose of according to all regulations.





