P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 – Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

SensiTek HRP Anti-Rabbit Lab Pack

Species of Origin: Goat
Antigen Specificity: Anti-Rabbit
Preadsorbed Against: Human
Enzyme Conjugate: Horseradish Peroxidase
Chromogen Substrate: None Provided

Uses/Limitations: Do not use past expiration date.
For immunohistochemical studies.

Availability:

REF #	Volume
SHR125	125ml Super Block, 125ml SensiTek Anti-Rabbit, 125ml SensiTek HRP.
SHR500	500ml Super Block, 500ml SensiTek Anti-Rabbit, 500ml SensiTek HRP.
SHR999	1000ml Super Block, 1000ml SensiTek Anti-Rabbit, 1000ml SensiTek HRP.

Storage: 2-8º Centigrade.

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in Tris Buffered Saline + Tween 20 (20X) pH 7.4 (catalog # TBT500).
3. If required, incubate tissue in digestive enzyme (catalog # PSS060 or TSS155) or Citrate Plus (catalog # CPL500).
4. Wash 3 times in Tris Buffered Saline.
5. 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.
7. Apply primary antibody and incubate according to manufacturer's protocol.
8. Wash 3 times in Tris Buffered Saline.
9. Apply SensiTek Anti-Rabbit (yellow solution), and incubate for 20 minutes at room temperature.
10. Wash 3 times in Tris Buffered Saline.

Storage: 2° C  8° C


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11. Apply SensiTek HRP (red solution), and incubate for 20 minutes at room temperature.
12. Wash 2 times in Tris Buffered Saline.
13. Rinse 1 time in DI/Distilled water.
14. Apply chromogen intended for use with Horseradish Peroxidase and incubate as desired.
15. For optimal results counterstain using Hematoxylin, Mayer's (catalog # HMM500).
16. 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.
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.

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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.
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.