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

Retrieval Lab Pack (Anti-Polyvalent / HRP)Ready-To-Use

Species of Origin: Goat
Antigen Specificity: Anti-Mouse, Rat, Rabbit, Guinea Pig
Preabsorbed Against: Human
Enzyme Conjugate: Peroxidase
Chromogen Substrate: None provided

Contents:
Citrate Plus (10X) HIER Solution (pH 6.0)
Retrieval Super Block
Retrieval Anti-Polyvalent, Biotinylated Antibody
Retrieval HRP

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. In a plastic Coplin jar, add 5 ml of Citrate Plus and 45 ml of deionized water.
3. Loosely cap the coplin jar and place in a vegetable steamer for 15 minutes to heat solution (Prior to submersion of slides).
4. Using tongs, remove the coplin jar from the steamer. Carefully remove cap and submerge slides. Recap loosely and return jar to steamer.
5. Steam for 20 minutes. Allow jar with solution and slides to cool to room temperature.
6. Remove slides and rinse in buffer several times.
7. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
8. Wash 2 times in buffer.
9. If required, incubate tissue in digestive enzyme.
10. Wash 4 times in buffer.
11. Apply Retrieval Super Block and incubate for 5-10 minutes at room temperature to block nonspecific background staining. *Note: Do not exceed 10 minutes or there may be a reduction in desired stain.*
12. Wash 1 time in buffer.
13. Apply primary antibody and incubate according to manufacturer's protocol.
14. Wash 4 times in buffer.
15. Apply Retrieval Anti-Polyvalent, Biotinylated Antibody and incubate for 10 minutes at room temperature.

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16. Wash 4 times in buffer.

17. Apply Retrieval HRP, and incubate for 10 minutes at room temperature.

13. Rinse 4 times in buffer.

14. Apply chromogen intended for use with Peroxidase (ie. DAB catalog# ACK500 or AEC catalog# ACJ500).

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 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.
7. Excessive incubation with protein block (Retrieval Super Block or normal serum).

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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.