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

SensiTek HRP Anti-Polyvalent (DAB) Ready-To-Use (70 slide)

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

Procedure:

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 5-10 minutes at room temperature to block nonspecific background staining. *Note: Do not exceed 10 minutes or there may be a reduction in desired stain.*
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 Biotinylated Link Antibody (yellow cap), and incubate for 15-20 minutes at room temperature.
11. Wash 4 times in buffer.
12. Apply Streptavidin/HRP Label (red cap), and incubate for 20 minutes at room temperature.
13. Rinse 4 times in buffer.
14. Add 4 drops (200ul) DAB Chromogen to DAB Substrate, mix by swirling and apply to tissue. Incubate for 5-15 minutes, depending on the desired stain intensity.
    WARNING:DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.
15. Counterstain and coverslip

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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. Room temperature was excessively cool.
5. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
6. 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 mouse, rat, rabbit or guinea pig origin.
4. Chromogenic substrate has been replaced with another that is not intended for use with peroxidase.