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

Rabbit-To-Rabbit Blocking Reagent

Product Description: ScyTek's Rabbit-to-Rabbit reagent has been formulated to provide the researcher with a staining system capable of visualizing Rabbit antibodies on Rabbit tissue. In most cases a 30-minute incubation with Rabbit-to-Rabbit block will virtually eliminate background staining that is caused by endogenous immunoglobulins. We highly recommend that this reagent be used in conjunction with ScyTek's UltraTek Anti-Rabbit staining system for optimal results.

Species of Origin: Goat
Antigen Specificity: Anti-Rabbit
Enzyme Conjugate: None
Chromogen Substrate: None

Procedure:

1. Deparaffinize and rehydrate tissue section.
2. If required 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 (ScyTek catalog# AAA), 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 Rabbit-To-Rabbit Block and incubate 10-60 minutes. Incubation time is dependent on the amount of endogenous Ig found in the tissue type.
9. Rinse 4 times in buffer.
10. Apply primary antibody and incubate according to manufacturer's protocol.
12. Wash 4 times in buffer.
13. Apply UltraTek Anti-Polyvalent (ScyTek catalog# ABN), and incubate for 10-20 minutes at room temperature.
14. Wash 4 times in buffer.

Storage: 2°C – 8°C

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15. Apply UltraTek HRP (ScyTek catalog# ABL) or UltraTek Alk-Phos (ScyTek catalog# ABM), and incubate for 10-20 minutes at room temperature.

16. Rinse 4 times in buffer.

17. Apply appropriate chromogen.

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 UltraTek Anti-Polyvalent , UltraTek HRP, or UltraTek Alk-Phos 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. Inadequate blocking with Rabbit-To-Rabbit Block.
5. Tissue contains endogenous biotin.
6. Antigen migrated in tissue.
7. Excessive tissue adhesive on slides.
8. Inadequate blocking with Super block.

Weak Staining:

1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents are past their expiration date.

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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.
7. Excessive incubation with 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 the correct species of origin.
4. Chromogenic substrate is not intended for use with enzyme used for procedure (peroxidase or alkaline-phosphatase).
5. One or more components of the kit have been inactivated by heat or other adverse condition.