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Product Name: Mouse X Rabbit Double Stain Kit (With DAB Brown/ HRP Green)

Catalog No.: TADS01

INTENDED USE: For Research Use Only. Not for use in diagnostic procedures.

DESCRIPTION: The double stain kits apply IHC technology and detect more than one target protein on the same section slide. The kit is an HRP detection system are those suitable for mouse and rabbit IgG and IgM primary antibodies. In this system, the powerful Immunoblock could reduce the background and blocking endogenous immunoglobulin at once time. The Systems included DAB Brown and HRP Green Chromogen. They will well define clearly two biomarkers as present in the same or different area.

APPLICATIONS: Immunohistochemistry (IHC)

Double stain kit contents with DAB Brown

Kit Contents	Format	Recommend time
Hydrogen Peroxide Block I	Ready to Use, 15ml	10-20 minutes.
Hi-effect Immunoblock I	Ready to Use, 15ml	10-30 minutes.
Mouse Probe HRP labeling I	Ready to Use, 15ml	30-45 minutes.
DAB Chromogen (20x)	Concentrate, 1ml	Diluted in DAB Buffer
DAB Buffer	Ready to Use, 15ml	1-10 minutes.

Double stain kit contents with HRP Green

Kit Contents	**Format**	**Recommend time**
Hydrogen Peroxide Block II	Ready to Use, 15ml	10-20 minutes.
Hi-effect Immunoblock II	Ready to Use, 15ml	10-30 minutes.
Rabbit Probe HRP Labeling II	Ready to Use, 15ml	30-45 minutes.
HRP Green Chromogen	Concentrate, 7.5ml	Diluted in HRP Green Buffer
HRP Green Buffer	Ready to Use, 7.5ml	5-15 minutes.

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STAINING PROTOCOL:

1. Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.
2. Add enough drops of Hydrogen Peroxide Block I to cover the sections. Incubate for 10minutes. Wash 2 times in PBS buffer.
3. Perform appropriate pretreatment if required. Wash 3 times in PBS buffer.
4. Apply Hi-effect Immunoblock I and incubate for 30 minutes to block non-specific background staining. Wash 2 times in PBS buffer.
5. Apply mouse primary antibody to the sections and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer to stop the binding.
6. Apply Mouse Probe HRP Labeling and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
7. Add 50 µl DAB Chromogen to 1.0 ml of DAB Buffer, mix by swirling and apply to tissue. Incubate for 1-10 minutes and observe in microscopy. Rinse 4 times in PBS buffer.
8. Add enough drops of Hydrogen Peroxide Block II to cover the sections. Incubate for 30 minutes. Wash 2 times in PBS buffer.
9. Apply Hi-effect Immunoblock II and incubate for 30 minutes to block non-specific background staining. Wash 2 times in PBS buffer.
10. Apply another rabbit primary antibody to the sections and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
11. Apply Rabbit Probe HRP labeling and incubate for 30 minutes at room temperature.
12. Wash 3 times in PBS buffer. Mix equal volume HRP Green Chromogen and HRP Green Buffer and apply to sections. Incubate for 5-15 minutes and observe in microscopy.
13. Wash 3 times in PBS buffer and rinse in tap water.
14. Apply hematoxylin to the section for countstain.
15. Running tap water wash for 5 minutes.
16. Air dry if required and coverslip. ＊Do NOT Dehydrate in alcohol, and Do Not use aqueous mounting.

STORAGE & STABILITY:

Store at 2-8°C. Do not freeze. The user must validate any other storage conditions. The reagents must be returned to the storage conditions identified above immediately after use. When properly stored, the reagent is stable to the date indicated on the label. Do not use the reagent beyond the expiration date. There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be tested simultaneously with unknown specimens. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the reagent is suspected, contact Technical Support at info@biotna.net or your distributor service.