BIOTnA Biotech
BIOSCIENCE FOR THE LAB.

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

Catalog No. : TADS03

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 HRP detection system those are 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)

Kit Contents	Format	Recommend time
Hydrogen Peroxide Block	Ready to Use	10-20 minutes.
Hi-effect Immunoblock	Ready to Use	10-30 minutes.
Mouse/Rabbit Probe HRP labeling	Ready to Use	30-45 minutes.
DAB Chromogen (33x)	Concentrate	Diluted in DAB Buffer
DAB Buffer	Ready to Use	1-10 minutes.
HRP Green Chromogen	Concentrate	Diluted in HRP Green Buffer
HRP Green Buffer	Ready to Use	5-15 minutes.

STAINING PROTOCOL:

1. Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.
2. Add enough drops of Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes. Wash 2 times in PBS buffer.
3. Perform appropriate pretreatment if required. Wash 3 times in PBS buffer.
4. Apply Hi-effect Immunoblock and incubate for 10 to 30 minutes to block non-specific background staining. (Depend on different tissue to adjust and raise the temperature to 37°C or reaction time can enhance the block effect.) Wash 2 times in PBS buffer.

5. Apply primary antibody and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
6. Apply Mouse/Rabbit Probe HRP Labeling and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
7. Add 30 µl (1 drop) DAB Chromogen to 1.0 ml (33 drops) of DAB Buffer, mix by swirling and apply to tissue. Incubate for 1-10 minutes. Rinse 4 times in PBS buffer.
8. Add enough drops of Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes. Wash 2 times in PBS buffer.
9. Apply Hi-effect Immunoblock and incubate for 10 to 30 minutes to block non-specific background staining. (Depend on different tissue to adjust and raise the temperature to 37°C or reaction time can enhance the block effect.) Wash 2 times in PBS buffer.
10. Apply primary antibody and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
11. Apply Mouse/Rabbit Probe HRP labeling and incubate for 30 minutes at room temperature.
12. Apply counterstain according to manufacturer's instructions (optional).
13. Wash 3 times in PBS buffer. Mix equal volume HRP Green Chromogen and HRP Green Buffer. Incubate for 5-15 minutes. Wash 3 times in PBS buffer.
14. Air dry if required and coverslip. ＊Do NOT Dehydrate, 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@tnabio.com or your distributor service.