BIOTnA Biotech

BIOSCIENCE FOR THE LAB.

Product No. : Rat X Mouse/Rabbit Double Stain Kit (With DAB Brown/ HRP Green}Catalog No. : TADS04

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

DESCRIPTION: The reagents in this kit constitute a biotin-free immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a secondary antibody- HRP conjugate which reacts with the primary antibody, and substrate-chromogen (DAB) and HRP Green.

APPLICATIONS: Immunohistochemistry (IHC)

Double stain kit contents with DAB Brown

Kit Contents	Format	Recommend time
Hydrogen Peroxide Block	Ready to Use	10-20 minutes.
Hi-effect Immunoblock	Ready to Use	10-30 minutes.
Rat probe	Ready to Use	10 minutes.
Rat 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.

Double stain kit contents with HRP Green

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.
HRP Green Chromogen	Concentrate	Diluted in HRP Green Buffer
HRP Green Buffer	Ready to Use	5-15 minutes.

BIOTnA Biotech

BIOSCIENCE FOR THE LAB.

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 rat primary antibody and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
6. Apply Rat Probe and incubate for 10 minutes at room temperature. Wash 3 times in PBS buffer.
7. Apply Rat Probe HRP labeling and incubate for 30 minutes at room temperature.Wash 3 times in PBS buffer.
8. 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. Wash 3 times in PBS buffer.
9. Add enough drops of Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes. Wash 2 times in PBS buffer.
10. 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.
11. Apply primary antibody and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
12. Apply Mouse/Rabbit Probe HRP labeling and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
13. Apply counterstain according to manufacturer's instructions (optional).
14. 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.
15. 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.