

Product No. : Mouse/Rabbit X Mouse/Rabbit Double Stain Kit (With AEC Solution / HRP Green)

Catalog No. : TADS03-AG

**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 AEC Solution and HRP Green Chromogen. They will well define clearly two biomarkers as present in the same or different area.

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.
AEC Soultion	Ready to Use	5-10 minutes.
HRP Green Chromogen	Concentrate	Diluted in HRP Green Buffer
HRP Green Buffer	Ready to Use	5-15 minutes.

**APPLICATIONS**: Immunohistochemistry (IHC)

## STAINING PROTOCOL:

- 1. Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.
- 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.



- 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.
- Add enough drops of AEC solution to cover the sections. Incubate for 5 -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.
- 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).
- 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 <u>info@tnabio.com</u> or your distributor service.