



Product Name: Immuno-Fluorescence multiple Stain Kit
(conjugated FAM/ TAMRA/Cy5)

Catalog No: TATS01F

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

DESCRIPTION: This multiple stain kit applies immuno-fluorescence technology and detect more than one target protein on the same tissue section. The kit is fluorescence detection system those are suitable for mouse and rabbit source IgG primary antibodies. A powerful reagent in this system, immunoblock could reduce the background from endogenous immunoglobulin and unbound primary antibody at once time. The signal included FAM (488), TAMRA (594) and Cy5 (680) dye conjugated secondary antibodies. They will well clearly define more than two biomarkers as present in the same or different area.

Kit Contents	Format	Recommend time	Storage
Immunoblock	Ready to Use, 45ml	30-60 minutes.	2-8°C
Mouse enhancer post primary antibody (Mouse enhancer)	Ready to Use, 45ml	15 minutes.	2-8°C
Anti-Rabbit IgG-FAM	Ready to Use, 15ml	30 minutes.	2-8°C
Anti-Rabbit IgG-TAMRA	Ready to Use, 15ml	30 minutes.	2-8°C
Anti-Rabbit IgG-Cy5	Ready to Use, 15ml	30 minutes.	2-8°C
DAPI Solution	Ready to Use, 15ml	1 minute.	2-8°C
Aqua mounting medium	Ready to Use, 15ml	---	25-28°C

STAINING PROTOCOL:

1. Normally process the rehydration for FFPE or frozen tissue sections.
2. Perform appropriate pretreatment if required. Wash 3 times in PBS buffer.
3. Apply Immunoblock and incubate for 30 minutes or more to block non-specific background. Wash 2 times in PBS buffer.
4. Apply mouse or rabbit primary antibody to the sections and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer to remove non-binding antibodies.
5. Apply Mouse enhancer post primary antibody to the sections as the mouse primary antibody used and incubate for 15 minutes at room temperature.
6. Apply Rabbit IgG-FAM to detect the Rabbit primary antibody and Mouse



- enhancer, incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
7. Apply Immunoblock again, incubate for 30-60 minutes to block non-specific background staining and unbound primary antibody. Wash 2 times in PBS buffer.
 8. Apply another mouse or rabbit primary antibody to the sections and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
 9. Apply Mouse enhancer again to the sections as the mouse primary antibody used and incubate for 15 minutes at room temperature.
 10. Apply Rabbit IgG-TAMRA and incubate for 30 minutes at room temperature. Wash 2 times in PBS buffer.
 11. Apply Immunoblock again, incubate for 30-60 minutes to block non-specific background staining and unbound primary antibody. Wash 2 times in PBS buffer.
 12. Apply the third mouse or rabbit primary antibody to the sections and incubate according to manufacturer's protocol. Wash 3 times in PBS buffer.
 13. Apply Mouse enhancer again to the sections as the mouse primary antibody used and incubate for 15 minutes at room temperature.
 14. Apply Rabbit IgG-Cy5 and incubate for 30 minutes at room temperature. Wash 2 times in PBS buffer.
 15. Apply DAPI Solution 1 minute for counterstain.
 16. Wash 2 times in RO water.
 17. Apply moderate aqua mounting medium to the slides and cover in coverslips.

STORAGE & STABILITY:

Store at 2-8°C but aqua mounting medium. 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 www.biotna.net or your distributor service.