



Real-one Rabbit IHC Staining Kit

Product Name: Real-one Rabbit IHC Staining Kit

Catalog No.: IRSK8002-15

Form: DAB Brown

Catalog No. Size	Size
IRSK8002-15	15ml / 150T

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 rabbit primary antibody, and substrate-chromogen (DAB).

APPLICATIONS: Immunohistochemistry (IHC)

KIT CONTENTS:	Description	Format	Recommend time
Hydrogen Peroxide Block	3% hydrogen peroxide solution with less than 0.1% sodium azide.	Ready to Use	10-20 minutes.
Immunoblock	PBS solution, pH 7.6, with 0.5% BSA, and less than 0.1% sodium azide.	Ready to Use	10-30 minutes.
Rabbit Probe HRP	Conjugated HRP Reactive with rabbit primary antibodies.	Ready to Use	30-45 minutes
DAB Chromogen (20x)	3,3' Diaminobenzidine (DAB) chromogen	Concentrate	Diluted with DAB Buffer (chromogen 1part : buffer 19 parts)
DAB Buffer	Substrate buffer, pH 7.5 with hydrogen peroxide.	Ready to Use	1-10 minutes.
Hematoxylin	Hematoxylin	Ready to Use	0.5-1 minutes.

Antigen retrieval buffer	20X Citrate Buffer (pH6)	20X	
Antibody dilution buffer	Primary antibody and incubate according to manufacturer's protocol	Ready to Use	

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 Immunoblock and incubate for 10-30 minutes at room temperature to block non-specific background staining. Wash 2 times in PBS buffer.
5. Apply rabbit primary antibody and incubate according to manufacturer's protocol.
6. Wash 3 times in PBS buffer.
7. Apply Rabbit Probe HRP and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
8. Add 50 µl DAB Chromogen to 950ul of DAB Buffer (20X dilution), mix by swirling and apply to tissue. Incubate for 1-10 minutes. Rinse 4 times in PBS buffer.
9. Rinse slide in tap water.
10. Apply Hematoxylin counterstain according to manufacturer's instructions (optional).
11. Dehydrate if required and cover slips.

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 irealbio@irealbio.com or your distributor service.

