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RhoA/B/C Ready-To-Use IHC Kit

Cat.No: IHC0399
Applications: **IHC-P**
Reactivity: Human, Mouse, Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (RhoA/B/C Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (human brain, rat brain, mouse brain)	3 slides	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **RhoA/B/C Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

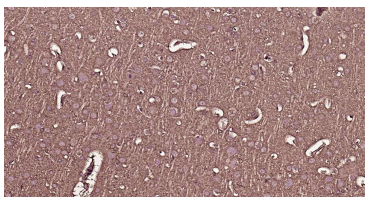
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0399, Bioss Antibodies". Citation example: " Tissue sections using RHOA IHC Kit (IHC0399, Bioss Antibodies) were stained for RHOA according to the manufacturer's instructions."

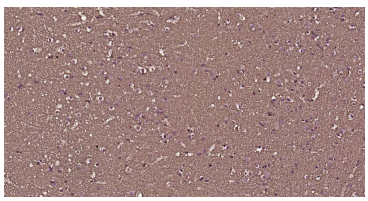
Introduction:

RhoA regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers. It is involved in a microtubule-dependent signal that is required for the myosin contractile ring formation during cell cycle cytokinesis, and plays an essential role in cleavage furrow formation. RhoA is required for the apical junction formation of keratinocyte cell-cell adhesion, and serves as a target for the yopT cysteine peptidase from Yersinia pestis, vector of the plague, and Yersinia pseudotuberculosis, which causes gastrointestinal disorders. RhoA stimulates PKN2 kinase activity and may be an activator of PLCE1. It is activated by ARHGEF2, which promotes the exchange of GDP for GTP. It is essential for the SPATA13-mediated regulation of cell migration and adhesion assembly and disassembly. The MEMO1-RHOA-DIAPH1 signaling pathway plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. It controls the localization of APC and CLASP2 to the cell membrane, via the regulation of GSK3B activity. In turn, membrane-bound APC allows the localization of the MACF1 to the cell membrane, which is required for microtubule capture and stabilization.

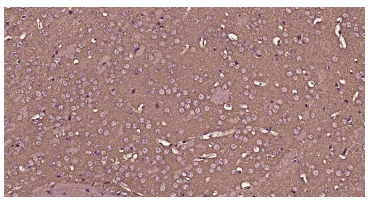
Validation Data



Immunohistochemical analysis of paraffin embedded rat brain tissue slide using IHC0399 (RhoA/B/C IHC Kit).



Immunohistochemical analysis of paraffin embedded human brain tissue slide using IHC0399 (RhoA/B/C IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse brain tissue slide using IHC0399 (RhoA/B/C IHC Kit).