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Human CA9 Ready-To-Use IHC Kit

Cat. No.: IHC0199H
Sample Type: FFPE tissue **Size: 50T**
(including a control slide)
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.

General Information

Number	Component	50T	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Human CA9 Mouse mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (AffiniPure Goat Anti-Mouse IgG H&L / HRP)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Human fundus of stomach)	1 slide	RTU	RT
13	Datasheet	1 copy		

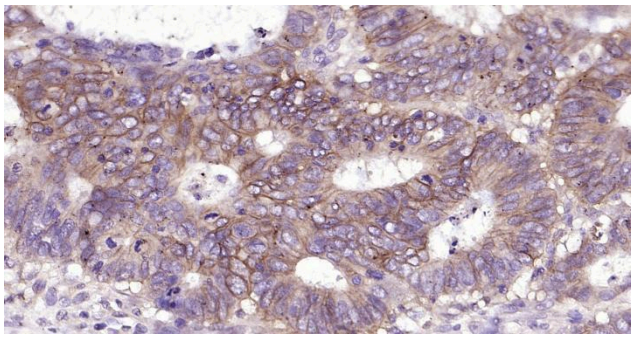
Background

Carbonic anhydrase (CA) is an enzyme that assists rapid interconversion of carbon dioxide and water into carbonic acid, protons, and bicarbonate ions. It is abundant in all mammalian tissues. There are many genes that are inducible by hypoxia, via HIF-1 alpha. CA IX is one of the most inducible genes because of its stability and location within the membrane. Carbonic anhydrases have a widespread role in regulating pH in normal tissues, by regulating hydrogen ion (H+) flux. The pH is important in cell death under hypoxia, thus a blockade of CA IX results in increased cell death under hypoxia. Therefore, CA IX has become a reliable histochemical marker of hypoxia.

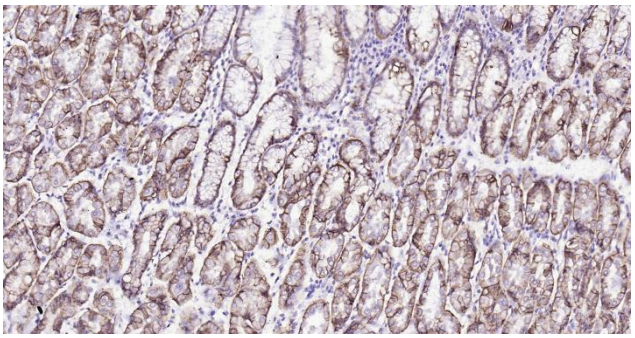
Synonyms

CAIX; G250; MN; CA IX; CA-IX; Carbonic anhydrase 9; CAH9_HUMAN; Carbonate dehydratase IX; Carbonic anhydrase IX; Carbonic dehydratase; Membrane antigen MN; P54/58N; P54/58N; pMW1; RCC associated protein G250; RCC-associated antigen G250; Renal cell carcinoma associated antigen G250; Renal cell carcinoma-associated antigen G250.

Validation Data



Immunohistochemical analysis of paraffin embedded human colon cancer tissue slide using IHC0199H (Human CA9 IHC Kit).



Immunohistochemical analysis of paraffin embedded human stomach tissue slide using IHC0199H (Human CA9 IHC Kit).

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 **Human CA9 Mouse 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 **AffiniPure Goat Anti-Mouse IgG H&L / HRP** 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 and Slides Mounting

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℃ 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 "IHC0199H, Bioss Antibodies". Citation example: “Human tissue sections using Human CA9 IHC Kit (IHC0199H, Bioss Antibodies) were stained for CA9 according to the manufacturer's instructions.