

## Human CK18 Ready-To-Use IHC Kit

Cat.No: IHC0249H  
Applications: **IHC-P**  
Reactivity: Human  
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 (Human CK18 Mouse mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (AffiniPure Goat Anti-Mouse 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 pancreatic cancer)	1 slide	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 **Human CK18 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 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.

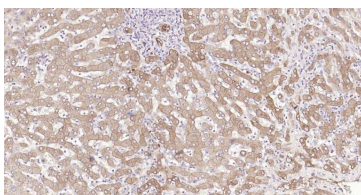
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

***Please cite this product as "IHC0249H, Bioss Antibodies". Citation example: "Human Tissue sections using KRT18 IHC Kit (IHC0249H, Bioss Antibodies) were stained for KRT18 according to the manufacturer's instructions."***

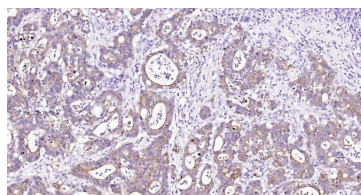
## Introduction:

Cytokeratin 18 (Keratin 18, KRT18, type I cytoskeletal 18) belongs to the type A (acidic) subfamily of low molecular weight keratins, exists in combination with cytokeratin 8. Tissues from gastrointestinal tract, respiratory tract and urogenital tract, as well as endocrine and exocrine tissues and mesothelial cells are positive for cytokeratin 18. Further, cytokeratin 18 is found primarily in non-squamous epithelia and is present in a majority of adenocarcinomas and ductal carcinomas, but not in squamous cell carcinomas. Hepatocellular carcinomas express only cytokeratins 8 and 18. Mutations in the cytokeratin 18 gene have been linked to cryptogenic cirrhosis. Two transcript variants encoding the same protein have been found for the cytokeratin 18 gene. Cytokeratin subtype expression patterns are used to an increasing extent in the distinction of different types of epithelial malignancies. The cytokeratin antibodies are not only of assistance in the differential diagnosis of tumors using immunohistochemistry on tissue sections, but are also a useful tool in cytopathology and flow cytometric assays.

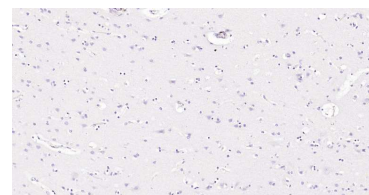
## Validation Data



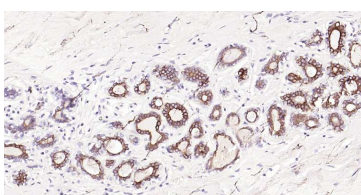
Immunohistochemical analysis of paraffin embedded human liver tissue slide using IHC0249H (Human CK18 IHC Kit).



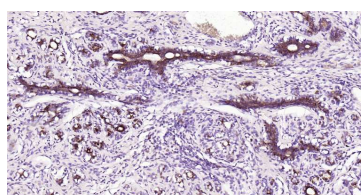
Immunohistochemical analysis of paraffin embedded human colon cancer tissue slide using IHC0249H (Human CK18 IHC Kit).



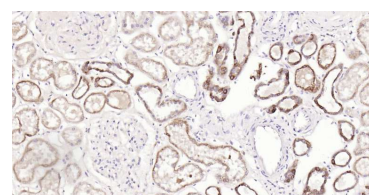
Immunohistochemical analysis of paraffin embedded human brain tissue slide using IHC0249H (Human CK18 IHC Kit).



Immunohistochemical analysis of paraffin embedded human mammary gland tissue slide



Immunohistochemical analysis of paraffin embedded human breast cancer tissue slide



Immunohistochemical analysis of paraffin embedded human kidney tissue slide using

using IHC0249H (Human CK18 IHC Kit).

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