Mouse pan CK Ready-To-Use IHC Kit

Cat #: orb1974481 (manual)

Size: 50T

For research use only. Not intended for diagnostic use.

Product Features

Applications: IHC-P

Sample Types: FFPE tissue

Storage: Please store components at the temperatures written in Kit Components. The kit is stable for 6 months from the date of receipt.

Background:

Cytokeratin pan Introduction

Cytokeratin pan comprises a diverse family of intermediate filament proteins expressed in epithelial tissues, represented by at least 20 polypeptides numbered 1–20. These proteins range from 40–68 kDa in molecular weight and have isoelectric points between pH 4.9 and 7.8. Specific cytokeratin expression patterns reflect epithelial type, differentiation, and maturation. Because these patterns vary with cell type, growth conditions, and disease state, cytokeratin subtypes are widely used to distinguish epithelial malignancies. Cytokeratin antibodies are valuable in immunohistochemistry, cytopathology, flow cytometry, and cancer diagnosis.

Kit Components

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 (Mouse pan CK Mouse mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (HRP-Goat anti-Mouse IgG pAb)	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

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11	Control slide (Mouse skin)	1 slide	RTU	RT
12	Datasheet	1 copy		

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 **Mouse pan CK 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 **HRP-Goat anti-Mouse IgG pAb** 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.

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Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.

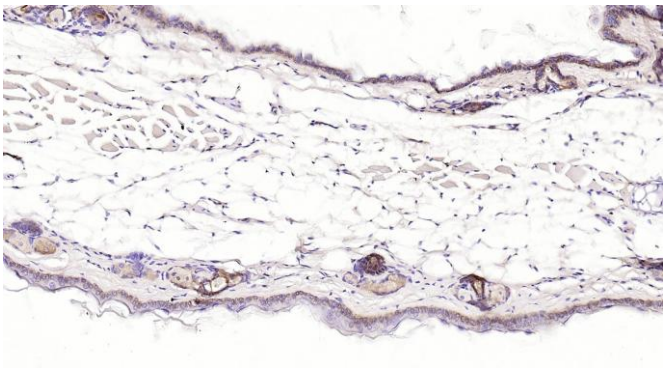
2. Do not allow slides to dry at any time during this procedure.

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

4. 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.

Validation Data

The data is for reference only.



Immunohistochemical analysis of paraffin embedded mouse skin tissue slide using orb1974481 (Mouse pan CK Ready-To-Use IHC Kit).

Declaration

1. For research use only. Not intended for diagnostic use.
2. Please don't replace the matching reagents in this product with other manufacturers' products.

Safety Notes

Please wear the lab coat, mask and gloves to protect yourself during the assay.

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