www.bioss.com.cn
sales@bioss.com.cn
techsupport@bioss.com.cn
400-901-9800

Human CD45 Ready-To-Use IHC Kit

Cat.No: IHC0127H
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 CD45 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 tonsil)	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 (Pressure Cooker)**

Prepare a 1x antigen retrieval solution by diluting the 100x Antigen Retrieval Buffer using distilled water. Add the appropriate amount of 1x antigen retrieval solution into the pressure cooker and place a heat-resistant staining container filled with the same solution inside the cooker. Heat the solution to boiling with the lid of the pressure cooker rested on

top without being secured. Once it's boiling, transfer the slides from the distilled water to the staining container inside the pressure cooker. Follow the manufacturer’ s instructions to secure the lid of the pressure cooker. As soon as the cooker reaches full pressure, time three minutes. After three minutes, move the pressure cooker to an empty sink and cool it down by running cold water over the cooker. Once depressurized, open the lid and transfer the staining container with the slides to room temperature. After 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 CD45 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:

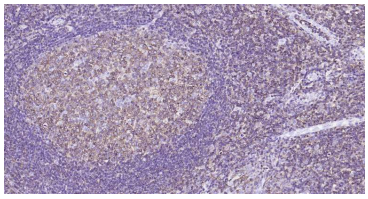
1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, 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 " IHC0127H, Bioss Antibodies". Citation example: " Human Tissue sections using PTPRC IHC Kit (IHC0127H, Bioss Antibodies) were stained for PTPRC according to the manufacturer's instructions."

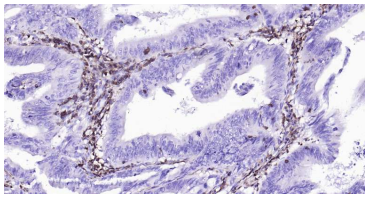
Introduction:

CD45 (LCA, leukocyte common antigen) is a receptor-type protein tyrosine phosphatase (PTP) ubiquitously expressed in all nucleated hematopoietic cells, comprising approximately 10% of all surface proteins in lymphocytes. CD45 is absent on non-hematopoietic cell lines, normal and malignant, non-hematopoietic tissues. CD45 glycoprotein is crucial in lymphocyte development and antigen signaling, serving as an important regulator of Src-family kinases. CD45 protein exists as multiple isoforms as a result of alternative splicing, differ in their extracellular domains but share identical transmembrane and cytoplasmic domains. CD45RA is an isoform of the CD45 complex and has restricted expression between different subtypes of lymphoid cells. CD45 isoforms differ in their ability to translocate into the glycosphingolipid-enriched membrane domains and their expression depends on cell type and physiological state of the cell. CD45 has been shown to be an essential regulator of T- and B-cell antigen receptor signaling and suppresses JAK kinases to regulate cytokine receptor signaling. CD45 is also important in promoting cell survival by modulating integrin-mediated signal transduction pathway, DNA fragmentation during apoptosis and inhibition or upregulation of various immunological functions.

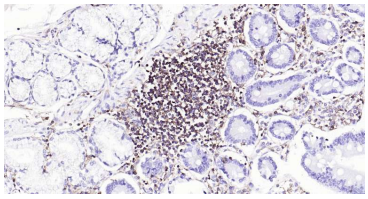
Validation Data



Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0127H (Human CD45 IHC Kit).



Immunohistochemical analysis of paraffin embedded human colon cancer tissue slide using IHC0127H (Human CD45 IHC Kit).



Immunohistochemical analysis of paraffin embedded human small intestine tissue slide using IHC0127H (Human CD45 IHC Kit).