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Human CD45 Ready-To-Use IHC Kit

Cat. No.: IHC0127H

Sample Type: FFPE tissue **Size: 50T (including 1 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	Size	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 CD45 Mouse mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Mouse IgG pAb)	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 tonsil)	1 slide	RTU	RT
13	Datasheet	1 copy		

Background

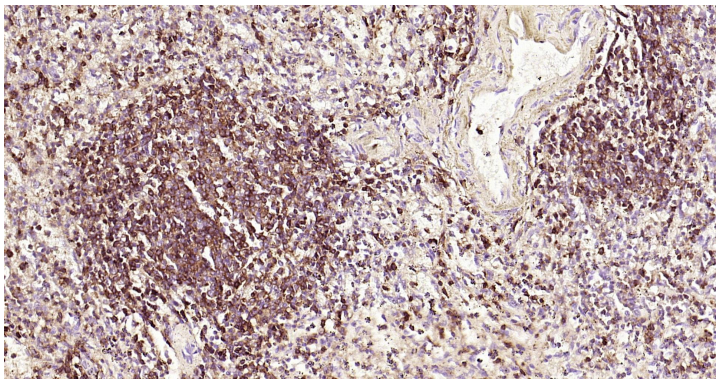
CD45 (LCA, leukocyte common antigen) is a receptor-type protein tyrosine phosphatase (PTP) ubiquitously expressed in all nucleated hematopoietic cells, comprising approximately 10% of all surface proteins in lymphocytes. CD45 is absent on non-hematopoietic cell lines, normal and malignant, non-hematopoietic 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.

Synonyms

B220; CD 45; CD-45; CD45; cd45 antigen; ec3.1.3.48; CD45R; GP180; GP180; GP 180; L CA; LCA; L-CA; Leukocyte common antigen; LY5; Ly-5 glycoprotein; Protein tyrosine phosphatase receptor type C; Protein

tyrosine phosphatase receptor type c polypeptide; protein tyrosine phosphatase, receptor type, C; Receptor-type tyrosine-protein phosphatase C; PTPRC; PTPRC_HUMAN; SCID due to PTPRC deficiency; T200; T200 glycoprotein; T200 leukocyte common antigen; Human homolog of severe combined immunodeficiency due to PTPRC deficiency.

Validation Data



Immunohistochemical analysis of paraffin embedded human spleen tissue slide using IHC0127H (Human CD45 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 CD45 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.

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 " IHC0127H, Bioss Antibodies". Citation example: "Human tissue sections using Human CD45 IHC Kit (IHC0127H, Bioss Antibodies) were stained for CD45 according to the manufacturer's instructions.