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Human CD8 Ready-To-Use IHC Kit

Cat. No.: IHC0190H
Sample Type: FFPE tissue (including a control slide) **Size: 50T**
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 CD8 Recombinant Rabbit mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Rabbit 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		

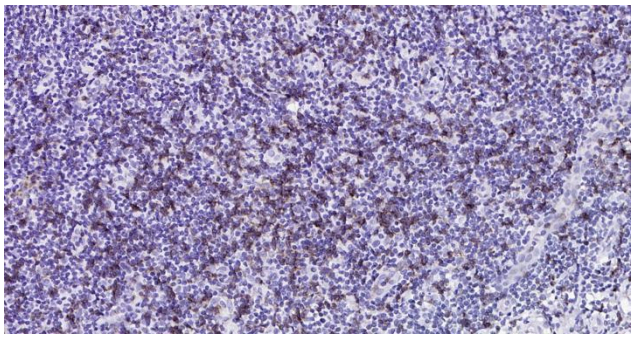
Background

Cluster of differentiation 8 (CD8), a type I transmembrane glycoprotein of the immunoglobulin family of receptors, plays an integral role in signal transduction, and T cell differentiation and activation. CD8 is predominantly expressed on T cells as a disulfide-linked heterodimer of CD8alpha and CD8beta, where it functions as a co-receptor, along with T cell receptor (TCR), for major histocompatibilty complex class I (MHC-I) molecules; whereas its counterpart, CD4, acts as a co-receptor for MHC-II molecules. CD8 exists on the cell surface, where the CD8alpha chain is essential for binding to MHC-I. CD8 is also expressed on a subset of T cells, NK cells, monocytes and dendritic cells as disulfide-linked homodimers of CD8alpha. Ligation of MHC-I/peptide complexes presented by antigen-presenting cells (APCs), triggers the recruitment of lymphocyte-specific protein tyrosine kinase (Lck), which leads to lymphokine production, motility and cytotoxic T lymphocyte (CTL) activation. Once activated, CTLs play a crucial role in the clearance of pathogens and tumor cells. Differentiation of naive CD8+ T cells into CTLs is strongly enhanced by IL-2, IL-12 and TGF-beta1.

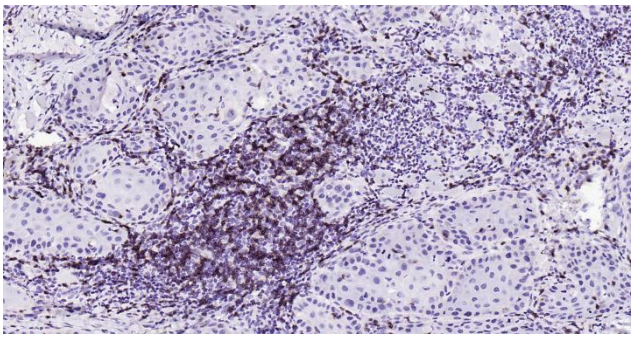
Synonyms

CD8, CD8A, CD8B, CD8B1, LY3, LYT3, MAL, P37, CD8 antigen, alpha polypeptide (p32), CD8a, CD8a antigen, CD8a molecule, CD8A_HUMAN, Leu2, Leu2 T lymphocyte antigen, MAL, OKT8 T cell antigen, p32, T cell antigen Leu2, T cell co receptor, T-cell surface glycoprotein CD8 alpha chain, T-lymphocyte differentiation antigen T8/Leu-2, T8 T cell antigen.

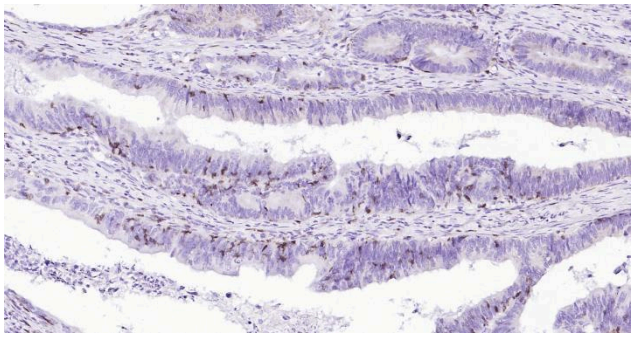
Validation Data



Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0190H (Human CD8 IHC Kit).



Immunohistochemical analysis of paraffin embedded human lung squamous cell carcinoma tissue slide using IHC0190H (Human CD8 IHC Kit).



Immunohistochemical analysis of paraffin embedded human colon carcinoma tissue slide using IHC0190H (Human CD8 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 (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 CD8 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 **HRP-Goat anti-Rabbit 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 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 "IHC0190H, Bioss Antibodies". Citation example: “Human tissue sections using Human CD8 IHC Kit (IHC0190H, Bioss Antibodies) were stained for CD8 according to the manufacturer's instructions.