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

Human CD25 Ready-To-Use IHC Kit

Cat.No: IHC0223H
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 CD25 Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L / HRP)	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**

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 CD25 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** 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.

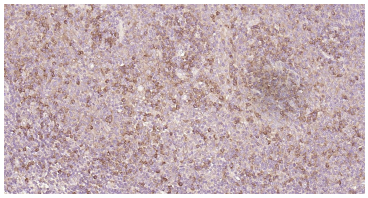
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0223H, Bioss Antibodies". Citation example: " Human Tissue sections using IL2RA IHC Kit (IHC0223H, Bioss Antibodies) were stained for IL2RA according to the manufacturer's instructions."

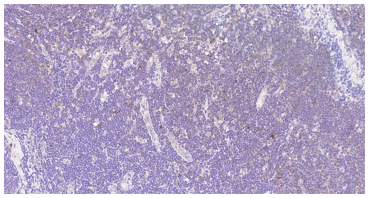
Introduction:

CD25 (IL2 receptor alpha chain/IL2RA) is a cytokine that plays a role in the proliferation of T and B lymphocytes. The receptor of this cytokine (IL2RA) is a heterotrimeric protein complex with a gamma chain also shared by interleukin 4 (IL4) and interleukin 7 (IL7). IL2RA, IL2R beta chain (IL2RB), and the IL2R gamma chain (IL2RG), constitute the high-affinity IL2 receptor. Homodimeric IL2RA chains result in low-affinity receptor, while homodimeric IL2RB chains produce a medium-affinity receptor. The expression of IL2 in mature thymocytes is monoallelic, which represents an unusual regulatory mode for controlling the precise expression of a single gene. IL2 is primarily produced by mature T cells. IL2 plays an important role as a growth factor, differentiation factor, and regulator of cell death. IL-2 stimulates the proliferation of B cells, augments natural killer cell activity, and inhibits granulocyte macrophage colony formation. The targeted disruption of a similar gene in mice leads to ulcerative colitis-like disease, which suggests a role in the immune response to antigenic stimuli. Mutations in this gene are associated with interleukin 2 receptor alpha deficiency.

Validation Data



Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0223H (Human CD25 IHC Kit).



Immunohistochemical analysis of paraffin embedded human lymph nodes tissue slide using IHC0223H (Human CD25 IHC Kit).