

Mouse CD163 Ready-To-Use IHC Kit

Cat.No: IHC0303M
Applications: **IHC-P**
Reactivity: Mouse
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 (Mouse CD163 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 (Mouse lung)	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 **Mouse CD163 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:

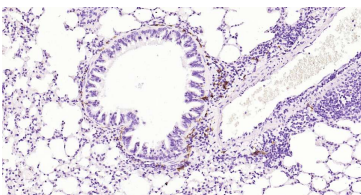
1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

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 "IHC0303M, Bioss Antibodies". Citation example: "Mouse Tissue sections using CD163 IHC Kit (IHC0303M, Bioss Antibodies) were stained for CD163 according to the manufacturer's instructions."

Introduction: CD163 (M130 antigen, Ber-Mac3, Ki-M8, SM4) is a 130 kDa membrane glycoprotein, a member of the scavenger receptor cysteine-rich superfamily, and a receptor for the hemoglobin-haptoglobin complex. CD163 protects tissues from free hemoglobin-mediated oxidative damage, and may play a role in the uptake and recycling of iron, via endocytosis of hemoglobin/haptoglobin and subsequent breakdown of heme. CD163 is expressed exclusively on the cell surface of human monocytes and macrophages that evolve predominantly in the late phase of inflammation. Specifically, CD163 is present on all circulating monocytes and most tissue macrophages except those found in the mantle zone and germinal centers of lymphoid follicles, interdigitating reticulum cells and Langerhan's cells. CD163 is present on all CD14 positive monocytes, most CD64 positive monocytes, and shows higher expression on CD16 positive monocytes. CD163 is upregulated on mononuclear phagocytes by IL-10, IL-6 and dexamethasone. Lipopolysaccharide (LPS) and phorbol myristate acetate (PMA) both induce shedding of CD163 from the cell surface into plasma or cell supernatant. CD163 binds hemoglobin/haptoglobin complexes in a calcium-dependent and pH-dependent manner, and exhibits a higher affinity for complexes of hemoglobin and multimeric haptoglobin of HP1F phenotype than for complexes of hemoglobin and dimeric haptoglobin of HP1S phenotype. Further, CD163 also induces a cascade of intracellular signals that involves tyrosine kinase-dependent calcium mobilization, inositol triphosphate production and secretion of IL6 and CSF1.

Validation Data



Immunohistochemical analysis of paraffin embedded mouse lung tissue slide using IHC0303M (Mouse CD163 Kit).