

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

Human CD68 Ready-To-Use IHC Kit

| Cat.No: | IHC0217H |
|---------------|----------------------|
| Applications: | ІНС-Р |
| 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 CD68 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 сору | | |

Storage andPlease store components at the temperatures indicated on the individual tube labels. TheStability:kit is stable for 6 months from the date of receipt.

Immunohistoche mistry 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 $100 \times$ **Antigen Retrieval Buffer** into distilled water to prepare a $1 \times$ solution. Boil slides in $1 \times$ solution at 95°C-100°C for 15 minutes. Move the slides to $1 \times$ 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 CD68 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.

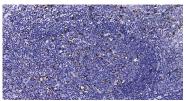
4. As DAB is a carcinogen, please take necessary precautions.

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

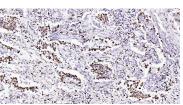
Please cite this product as "IHC0217H, Bioss Antibodies". Citation example: "Human Tissue sections using CD68 IHC Kit (IHC0217H, Bioss Antibodies) were stained for CD68 according to the manufacturer's instructions."

Introduction: CD68 (Macrosialin) is a 110 kDa integral membrane glycoprotein predominantly expressed on the intracellular lysomsomes of monocytes and macrophages and to a lesser extent by dendritic cells and peripheral blood granulocytes. Also, CD68 could play a role in phagocytic activities of tissue macrophages, both in intracellular lysosomal metabolism and extracellular cell-cell and cell-pathogen interactions. CD68 is expressed by interdigitating reticulum cells in tonsil and some histiocytic lymphoma or histiocytosis, acute myeloid leukemia (AML), and granulocytic sarcoma. Elevated expression of CD68 has been demonstrated on CD34+ cells in various human malignancies, including several Acute Myeloid Leukemia studies.

Validation Data



Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0217H (Human CD68 IHC Kit).



Immunohistochemical analysis of paraffin embedded human lung cancer tissue slide using IHC0217H (Human CD68 IHC Kit).



Immunohistochemical analysis of paraffin embedded human endometrial carcinoma tissue slide using IHC0217H (Human CD68 IHC Kit).



Immunohistochemical analysis of paraffin embedded human liver tissue slide using IHC0217H (Human CD68 IHC Kit).



Immunohistochemical analysis of paraffin embedded human cervical cancer tissue slide using IHC0217H (Human CD68 IHC Kit).