# **Anti-CD163 Antibody [A3B3]**

#### EM1901-90



Product Type: Mouse monoclonal IgG1, primary antibodies

Species reactivity: Human

**Applications:** WB, IHC-P, FC

**Molecular Wt:** Predicted band size: 125 kDa.

Clone number: A3B3

**Description:** Acute phase-regulated receptor involved in clearance and endocytosis of hemoglobin/haptoglobin complexes

by macrophages and may thereby protect tissues from free hemoglobin-mediated oxidative damage. Exhibits a higher affinity for complexes of hemoglobin and multimeric haptoglobin of HP\*1F phenotype than for complexes of hemoglobin and dimeric haptoglobin of HP\*1S phenotype. Induces a cascade of intracellular signals that involves tyrosine kinase-dependent calcium mobilization, inositol triphosphate production and secretion of IL6 and CSF1. After shedding, the soluble form (sCD163) may play an anti-inflammatory role, and may be a valuable diagnostic parameter for monitoring macrophage activation in inflammatory conditions. Intravenous lipopolysaccharide (LPS) produces a rapid rise of sCD163 in plasma of patient as it induces metalloproteinase-mediated shedding from monocytes surface. The soluble form (sCD163) in plasma is a novel parameter in diseases affecting macrophage function and monocyte/macrophage load in the body. The concentration of sCD163 is probably reflecting the number of macrophages of the 'alternative macrophage activation' phenotype with a high CD163 expression playing a major role in dampening the inflammatory response and scavenging components of damaged cells. This has initiated a number of clinical studies for evaluation of sCD163 as a disease marker in inflammatory conditions e.g. infection, autoimmune disease, transplantation, atherosclerosis

and cancer.

**Immunogen:** Recombinant protein within human CD163 aa 1-170.

Positive control: Human lung tissue lysate, human liver tissue lysate, human lung tissue, human liver carcinoma tissue, human

colom tissue, human placenta tissue, HT-29.

**Subcellular location:** Secreted, cell membrane.

Database links: SwissProt Q86VB7 Human

**Recommended Dilutions:** 

 WB
 1:500-1:2,000

 IHC-P
 1:100-1:500

 FC
 1:50-1:100

Storage Buffer: 1\*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% SodiumAzide.

**Storage Instruction:** Store at +4  $^{\circ}$ C after thawing. Aliquot store at -20  $^{\circ}$ C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein G affinity purified.

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**Images** 

kDax x x 250-150-150-150-72-55-45-35-25-14**Fig1:** Western blot analysis of CD163 on different lysates with Mouse anti-CD163 antibody (EM1901-90) at 1/1,000 dilution.

Lane 1: Human lung tissue lysate Lane 2: Human liver tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 125 kDa Observed band size: 150-170 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (EM1901-90) at 1/1,000 dilution was used in 5% NFDM/TBST at 4% overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

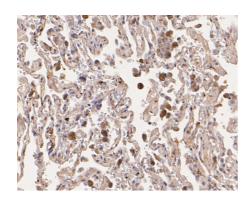
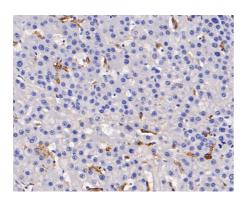


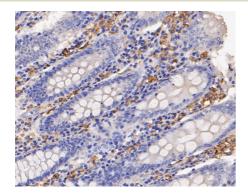
Fig2: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-CD163 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (EM1901-90, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



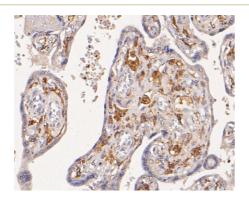
**Fig3:** Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue using anti-CD163 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (EM1901-90, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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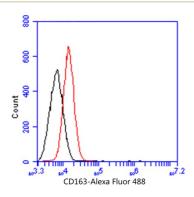




**Fig4:** Immunohistochemical analysis of paraffin-embedded human colom tissue using anti-CD163 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (EM1901-90, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-CD163 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (EM1901-90, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Flow cytometric analysis of CD163 was done on HT-29 cells. The cells were fixed, permeabilized and stained with the primary antibody (EM1901-90, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Akila P. et. al. Retraction Notice to "CD163 AND ITS EXPANDING FUNCTIONAL REPERTOIRE". Clin ChimActa. 2018 Dec.
- 2. Yang H. et al. Identification of CD163 as an antiinflammatory receptor for HMGB1-haptoglobin complexes. JCI Insight. 2018 Dec.

