Overview

Product name:
Anti-CD163 antibody

Description:
Rabbit polyclonal to CD163

Host species:
Rabbit

Tested applications:
Suitable for: IHC-P, WB, ICC/IF

Species reactivity:
Reacts with: Human

Predicted to work with: Pig, Chimpanzee, Macaque monkey, Gorilla, Orangutan

Immunogen:
Synthetic peptide conjugated to KLH derived from within residues 1050 - 1150 of Human CD163. Read Abcam's proprietary immunogen policy

Positive control:
WB: Human spleen and human thymus tissue lysates. IHC-P: Human spleen tissue sections. ICC/IF: Jeg3 cells.

Properties

Form:
Liquid

Storage instructions:
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer:
pH: 7.40
Preservative: 0.02% Sodium azide
Constituent: PBS

Note: Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity:
Immunogen affinity purified

Clonality:
Polyclonal

Isotype:
IgG

Applications

Our Abpromise guarantee covers the use of ab87099 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
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. May play a role in the uptake and recycling of iron, via endocytosis of hemoglobin/haptoglobin and subsequent breakdown of heme. Binds hemoglobin/haptoglobin complexes in a calcium-dependent and pH-dependent manner. 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. Isoform 3 exhibits the higher capacity for ligand endocytosis and the more pronounced surface expression when expressed in cells.

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.

Tissue specificity
Expressed in monocytes and mature macrophages such as Kupffer cells in the liver, red pulp macrophages in the spleen, cortical macrophages in the thymus, resident bone marrow macrophages and meningeal macrophages of the central nervous system. Expressed also in blood. Isoform 1 is the lowest abundant in the blood. Isoform 2 is the lowest abundant in the liver and the spleen. Isoform 3 is the predominant isoform detected in the blood.

Sequence similarities
Contains 9 SRCR domains.

Domain
The SRCR domain 3 mediates calcium-sensitive interaction with hemoglobin/haptoglobin complexes.

Post-translational modifications
A soluble form (sCD163) is produced by proteolytic shedding which can be induced by lipopolysaccharide, phorbol ester and Fc region of immunoglobulin gamma. This cleavage is dependent on protein kinase C and tyrosine kinases and can be blocked by protease inhibitors. The shedding is inhibited by the tissue inhibitor of metalloproteinase TIMP3, and thus probably induced by membrane-bound metalloproteinases ADAMs. Phosphorylated.

Cellular localization
Secreted and Cell membrane. Isoform 1 and isoform 2 show a lower surface expression when expressed in cells.

Application | Abreviews | Notes
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IHC-P | | Use a concentration of 1 - 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB | | Use a concentration of 1 - 2 µg/ml. Detects a band of approximately 150 kDa (predicted molecular weight: 125 kDa).
ICC/IF | | Use a concentration of 5 µg/ml.
IHC image of ab87099 staining in human spleen formalin fixed paraffin embedded tissue section*T, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab87099, 1µg/ml for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre
**Western blot - Anti-CD163 antibody (ab87099)**

- **All lanes**: Anti-CD163 antibody (ab87099) at 1 µg/ml
- **Lane 1**: Human spleen tissue lysate - total protein (ab29699)
- **Lane 2**: Human thymus tissue lysate - total protein (ab30146)

Lysates/proteins at 10 µg per lane.

**Secondary**

- **All lanes**: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 125 kDa

**Observed band size**: 150 kDa

**Additional bands at**: 12 kDa. We are unsure as to the identity of these extra bands.

**Exposure time**: 20 minutes

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**Immunocytochemistry/ Immunofluorescence - Anti-CD163 antibody (ab87099)**

ICC/IF image of ab87099 stained Jeg3 cells. The cells were 4% paraformaldehyde fixed (10 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab87099, 5µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
**Western blot - Anti-CD163 antibody (ab87099)**

- **All lanes**: Anti-CD163 antibody (ab87099) at 2 µg/ml (3% milk)

- **Lane 1**: Human thymus tissue lysate - total protein (ab30146)
- **Lane 2**: Human spleen tissue lysate - total protein (ab29699)

Lysates/proteins at 20 µg per lane.

**Secondary**

- **All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

- **Predicted band size**: 125 kDa
- **Observed band size**: 150 kDa
- **Additional bands at**: 35 kDa, 45 kDa, 55 kDa. We are unsure as to the identity of these extra bands.

**Exposure time**: 20 minutes

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**Please note**: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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