**Product datasheet**

**Anti-CD163 antibody [EPR19518] ab182422**

**Overview**

**Product name**
Anti-CD163 antibody [EPR19518]

**Description**
Rabbit monoclonal [EPR19518] to CD163

**Host species**
Rabbit

**Tested applications**
Suitable for: Flow Cyt, IHC-P, WB, IHC-Fr

**Species reactivity**
Reacts with: Mouse, Rat, Human

**Immunogen**
Recombinant fragment within Mouse CD163 aa 1-250. The exact sequence is proprietary.
Database link: Q2VLH6

**Positive control**
WB: Human fetal liver, spleen and tonsil lysates; Mouse and rat liver, heart, spleen and thymus lysates; U937, THP-1 and J774A.1 cell lysates IHC-P: Human liver, tonsil and placenta tissue.; human breast carcinoma tissue; Mouse liver and spleen tissue. Rat liver, achilles and muscle tissues; pig lung tissue; dog lymph node; IHC-Fr: Mouse spleen and liver tissues. FC: Human PBMC cells

**General notes**
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.
This product is a recombinant rabbit monoclonal antibody.

**Properties**

**Form**
Liquid

**Storage instructions**

**Storage buffer**
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

**Purity**
Protein A purified

**Clonality**
Monoclonal

**Clone number**
EPR19518

**Isotype**
IgG
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. 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.

**Applications**

Our Abpromise guarantee covers the use of ab182422 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Flow Cyt</td>
<td>1/60.</td>
<td></td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★☆</td>
<td>1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★☆</td>
<td>1/1000. Detects a band of approximately 150 kDa (predicted molecular weight: 121 kDa).</td>
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<tr>
<td>IHC-Fr</td>
<td>1/200.</td>
<td>Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).</td>
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</table>
Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on mouse spleen is observed. Counterstained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse spleen tissue labeling CD163 with ab182422 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result showed some cytoplasmic staining on mouse spleen. The nuclear counterstain is DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 at 1/1000 dilution. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Verified customer

10% NBF, non-permeabilized mouse spleen tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor®647 conjugate was used as the secondary at a 1/200 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Sodium citrate pH 6.0.

Blocking step: 5% serum for 1 hour at 21°C.

Flow cytometry analysis of human PBMC cells (Human peripheral blood mononuclear cell) labeling with ab182422 at 1/60 dilution, 11.23 μg/ml (red). Goat anti rabbit IgG (Alexa Fluor® 488, ab150081) was used as the secondary antibody at 1/2000.

All lanes: Anti-CD163 antibody [EPR19518] (ab182422) at 1/1000 dilution

Lane 1: Human fetal liver lysate
Lane 2: Human tonsil lysate
Lane 3: Human fetal spleen lysate
Lane 4: U937 (Human histiocytic lymphoma cell line) whole cell lysate
Lane 5: THP-1 (Human monocytic leukemia cell line) whole cell lysate
Lane 6: J774A.1 (Mouse macrophage reticulum cell sarcoma cell line) whole cell lysate

Lysates/proteins at 20 μg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 121 kDa
**Observed band size:** 150 kDa

*why is the actual band size different from the predicted?*

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 1 minute; Lane 2-5: 3 minutes.

U937, THP-1 and J774A.1 cell lines were reported to be negative for CD163 expression. (PMID:16368951, 10648003 & 10577520).

The molecular weight observed is consistent with what has been described in the literature (PMID:9712057 & 16517975).

10% NBF, non-permeabilized rat muscle tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor®647 conjugate was used as the secondary (red).

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 5% serum for 1 hour at 22°C.

10% NBF, non-permeabilized pig lung tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor®647 conjugate was used as the secondary at a 1/200 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 5% serum for 1 hour at 22°C.
Formaldehyde-fixed, non-permeabilized dog lymph node tissue stained for CD163 with ab182422 (60 mins at a 1/100 dilution) in immunohistochemical analysis. A Goat polyclonal HRP conjugate was used as the secondary.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Formaldehyde-fixed, non-permeabilized human tonsil tissue stained for CD163 with ab182422 (30 mins at a 1/400 dilution) in immunohistochemical analysis. A Goat polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 9.0 EDTA.

Blocking step: 1% ab64226 for 10 mins at RT.
Formalin-fixed, non-permeabilized human placenta tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Goat anti Rabbit polyclonal HRP conjugate was used as the secondary at a 1/200 dilution.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate buffer.

Blocking step: 3% serum for 30 mins at 20°C.

Formalin-fixed, non-permeabilized mouse liver tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Goat anti Rabbit HRP conjugate was used as the secondary at a 1/200 dilution.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate buffer.

Blocking step: 3% serum for 30 mins at 20°C.

10% Formalin-fixed, non-permeabilized human breast carcinoma tissue stained for CD163 with ab182422 (12 hours, 4°C at a 1/200 dilution) in immunohistochemical analysis. A Donkey anti Rabbit IgG polyclonal AlexaFluor®647 conjugate was used as the secondary at a 1/200 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 5% serum for 1 hour at 22°C.
Formaldehyde-fixed, non-permeabilized human first trimester placenta tissue stained for CD163 with ab182422 (16 hours, 4°C at a 1/250 dilution) in immunohistochemical analysis. A Pig anti Rabbit polyclonal biotin conjugate was used as the secondary at a 1/250 dilution.

Heat mediated antigen retrieval buffer/enzyme used: Sodium citrate.

Blocking step: 5% BSA for 30 mins at 22°C.

10% NBF-fixed mouse spleen tissue stained for CD163 with ab182422 (18 hours at a 1/250 dilution) in immunohistochemical analysis. A Goat anti Rabbit IgG polyclonal AlexaFluor®647 conjugate was used as the secondary at a 1/600 dilution (red).

Heat mediated antigen retrieval buffer/enzyme used: Tris/EDTA pH 9.0.

Blocking step: 20% serum for 1 hour at RT.
Formaldehyde-fixed, non-permeabilized mouse liver tissue stained for CD163 with ab182422 (45 mins at a 1/400 dilution) in immunohistochemical analysis. A Rabbit polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate.

Blocking step: 1% ab64226 for 10 mins at RT.

Formaldehyde-fixed, non-permeabilized rat achilles tissue stained for CD163 with ab182422 (30 mins at a 1/200 dilution) in immunohistochemical analysis. A Rabbit polyclonal HRP conjugate was used as the secondary.

Heat mediated antigen retrieval buffer/enzyme used: pH 6.0 citrate 70°C for 2hrs.

Blocking step: 1% ab64226 for 10 mins at RT.

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of human liver is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Hofbauer cells in human placenta is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of mouse liver is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Immunohistochemical analysis of formalin-fixed paraffin-embedded sections - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling CD163 with ab182422 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on Kupffer cells of rat liver is observed. Counter stained with Hematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Frozen sections) - Anti-CD163 antibody [EPR19518] (ab182422)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse liver tissue labeling CD163 with ab182422 at 1/200 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result showed some cytoplasmic staining on mouse liver. The nuclear counterstain is DAPI (blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab150077 at 1/1000 dilution. Antigen retrieval: Heated citrate solution (10mM citrate pH 6.0 + 0.05% Tween-20).

All lanes : Anti-CD163 antibody [EPR19518] (ab182422) at 1/1000 dilution

Lane 1 : Mouse liver lysate
Lane 2 : Mouse heart lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Mouse thymus lysate
Lane 5 : Rat liver lysate
Lane 6 : Rat heart lysate
Lane 7 : Rat spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary
**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size**: 121 kDa

**Observed band size**: 150 kDa

*why is the actual band size different from the predicted?*

**Exposure time**: 3 minutes

**Blocking/Dilution buffer**: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID:9712057 & 16517975).

**Please note**: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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