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# CD163 Enzyme Immunoassay

Test for the determination of CD163 in biological fluids Test Instructions

Product Code: S-1015

Kit contains:Precoated microtiter plate and reagents for 96 testsRefrigerate upon arrival, do not freeze.For in vitro research only.

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### **Introduction and Basic Information**

CD163 is known by several names: Scavenger Receptor, Cysteine Rich (SRCR); M130 protein; p155; haemoglobin scavenger receptor HbSR; RM3/1 antigen.

CD163 is expressed by monocytes and macrophages. These cells play a central role in the host response to infection. They synthesize and secrete a variety of inflammatory mediators. It has become increasingly clear that the pro-inflammatory process is balanced by concomitant anti-inflammatory mechanisms that result in monocyte deactivation, characterized by a decrease in HLA-DR expression and the release of anti-inflammatory cytokines such as interleukin (IL)-10. This counteraction is needed to control the extent of the inflammatory response. However, if prolonged or predominant, it may hamper the host's ability to react to inflammatory conditions. The histological distribution in inflamed tissues of various stages suggests a central role for CD163 in the complex network of interactions that lead to the resolution of inflammation.

#### Function of CD163

CD163 is a membrane protein. It is a member of the group B Scavenger Receptor Cysteine-Rich superfamily expressed on peripheral blood monocytes, and more strongly on most macrophages. It can be processed in two different ways: a) Upon appropriate stimulation (e.g. lipopolysaccharide, LPS or phorbol 12-myristate 13-acetate, PMA), the extracellular portion is shed from the cell surface in the form of soluble CD163 (sCD163). This process is protein kinase dependent and can be inhibited by tissue inhibitors of metalloproteinases (TIMPs), particularly TIMP-3. Serum levels of sCD163 have been shown to be associated with levels of CRP. Additionally, sCD163 acts as a cytokine with modulatory effects on other cells. b) CD163 mediates scavenging of haemoglobin through endocytosis of the CD163haptoglobin-haemoglobin complexes. CD163 binds only haemoglobin complexed to haptoglobin. The binding is Ca<sup>2+</sup> dependent and of high affinity. The CD163haptoglobin-haemoglobin complex is actively taken up by the involved cells. This process is therefore central for clearing toxic haemoglobin from the circulation, and for uptake of iron into the tissue macrophage. Cross-linking of CD163 has been shown to induce a protein tyrosine kinase dependent signal resulting in slow-type Ca<sup>2+</sup> mobilization, inositol trisphosphate production and secretion of IL-6 and GM-CSF.

The expression of CD163 is modulated by pro- and anti-inflammatory mediators. In particular, it is inducible by glucocorticoids and other anti-inflammatory reagents like IL-10. Glucocorticoids and IL-10 increase CD163 expression through independent pathways. Pretreatment of monocytes with dexamethasone increases expression of CD163 and adhesion to unstimulated and stimulated endothelial cells (HUVECs). On the other hand, cyclosporin A and pro-inflammatory mediators cause a down-regulation of the antigen. Treatment with cyclosporin A causes down-regulation of CD163 and also decreases adhesion to HUVECs. In contrast, the expression of other adhesion molecules remains unaffected by dexamethasone or Cyclosporin A. The combination of anti CD163 and anti CD14 inhibits adhesion of dexamethasone-induced monocytes to LPS- or IFN $\gamma$ -stimulated HUVECs. Thus, CD163 seems to contribute to the adhesion of cortisone-induced monocytes to LPS or cytokine-stimulated endothelial cells.

Histologically, CD163 positive macrophages are a major cell subpopulation in human term placenta. This finding may reflect a mechanism whereby placenta functions as an active immunosuppressive biological barrier between mother and fetus.

#### Structure of CD163

The extracellular region of the CD163 molecule contains nine repeating units. They each contain approximately 110 amino acids, characteristic of the scavenger receptor domain superfamily. Other members of this family include CD5 and CD6, complement factor I, the long form of the macrophage scavenger receptors, and the WC1 antigen in cattle. The scavenger receptor (Sc) domains in CD163 and WC1 show the greatest overall degree of sequence identity (46%). In addition, long-range repeating structures are found in both the CD163 and WC1 molecules. Several variants of CD163 mRNA are described. They encode proteins with altered cytoplasmic or extracellular domains and thus may differ in their functional properties. The gene is located on chromosome 12p13. Nucleotide analysis reveals several putative binding sites for transcription factors, which play an important role in myeloid specific gene expression.

#### Pathological significance

Intravascular haemolysis is a physiological phenomenon as well as a severe pathological complication when accelerated in various autoimmune, infectious (such as malaria) and inherited (such as sickle cell disease) disorders. Efficient removal of free haemoglobin is essential for health because of the oxidative and toxic properties of the iron-containing haem. Haemoglobin can also bind LPS and enhance its toxicity. Histologically, the presence of CD163 has been associated with a post-infectious recovery phase and declining inflammation. Measurements of circulating sCD163 levels have been published only recently. They have shown that sCD163 may be a valuable marker in diseases with macrophage / monocyte involvement, particularly in infectious and myeloproliferative diseases.

#### Selected Literature

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# **Test principle**

Three-step non-competitive sandwich assay, reagent limited with Peroxidase catalyzed Tetramethylbenzidine color reaction, including a stop reaction and reading at 450nm (reference wavelength: 620 - 650nm) in a multititer plate reader.

For Research use only, not for diagnostic application.

# **Reagents provided**

Catalog No.	Component	Quantity
S-1015A	Microtiter plate, coated with monoclonal anti CD163, ready	12 strips,
S 1015A2	lo use Plata scalar	
3-1015AZ		1
S-1015B 1-3	Standards (3) with 300ng/ml, 60ng/ml and 12ng/ml, ready to use	3 x 1.8ml
S-1015C	Detection antibody: polyclonal chicken anti human CD163 antibody. Red solution ready to use	22 ml
S-1015D	Assay buffer. Purple solution, ready to use	120 ml
S-1015E	Tetramethylbenzidine (TMB) - H <sub>2</sub> O <sub>2</sub> (see below)	1.25 ml
S-1015F	Substrate buffer (potassium citrate) (see below)	25 ml
S-1015H	Peroxidase conjugate: 100x concentrated peroxidase labeled anti chicken antibody. <i>(see below)</i>	0.3 ml
S-1015J	Wash buffer, 8-fold concentrated (see below)	125 ml

*Material not provided:* Plastic tubes for sample dilution. STOP solution (1N sulfuric acid, H<sub>2</sub>SO<sub>4</sub>), pipettes, microplate washer and reader with 450nm filter.

*Stability:* The kit is best used within the date indicated on the box. Store refrigerated, do not freeze.

## **Preparations**

Let all the reagents warm up to room temperature before starting, particularly the wash buffer concentrate (warm at 37°C to dissolve precipitate, if necessary). Duplicate testing of samples and standards is highly recommended. Prepare the following dilutions immediately before use:

**Samples:** Store serum samples in aliquots at  $-20^{\circ}$ C or lower. Preliminary tests have shown that samples can be frozen and thawed without adverse effects. Dilute your sample **1:200** in assay buffer (S-1015D). Example: add 5µl sample to 995µl assay buffer.

**Peroxidase conjugate:** A peroxidase coupled polyclonal antibody is supplied 100x concentrated. Dilute appropriately, e.g. by adding 0.2ml peroxidase conjugate to 20ml assay buffer (20ml are sufficient for 96 tests). The diluted solution is stable for several days when stored at  $4^{\circ} - 8^{\circ}$ C.

**TMB working solution:** Prepare immediately before use. Add  $80\mu$ I TMB -  $H_2O_2$  solution (S-1015E) to 1.7ml substrate buffer (S-1015F) **per strip** that is going to be used. Example: Add 1ml TMB -  $H_2O_2$  solution to 20ml substrate buffer if the whole plate is going to be used in one experiment. The diluted working solution should be used within 15 minutes after preparation.

#### Wash buffer:

Some reversible precipitation often occurs after storing the concentrate in the cold. **Check for complete dissolution**, warm up briefly if necessary. Dilute 1 part of the wash buffer concentrate with 7 parts distilled water: Example: add 125ml wash buffer concentrate to 875ml distilled water.

### STOP solution

Dilute sulfuric acid to a concentration of **1N**. Example: Add 2.9ml 95-97% sulfuric acid to 100ml water (In this sequence. Do not add the water to the acid). 95-97% concentrated sulfuric acid (specific gravity 1.84) is 36N.

# **Test procedure**

It is recommended to perform every measurement, including standards, in duplicate.

### 1. Antigen Binding

- 1a. Add  $200\mu$ l of each of the three standard solutions and one blank ( $200\mu$ l assay buffer; 0ng/ml) to their corresponding well.
- 1b. Add  $200\mu$ l sample (diluted 1:200 with assay buffer) to the corresponding wells.
- 1c. Seal plate and incubate 14 to 16 hours (over night) at 20-25°C (room temperature).

The incubation time may be reduced down to 5 hours at 20-25°C (room temperature). Absorbance values will be lower but the resulting sample values will not be significantly different.

### 2. Antigen detection

- 2a. Wash the plate 3 times with wash buffer, 250μl per well per washing. Blot onto a soft absorbing paper.
- 2b. Add 200µl detection antibody (red solution) per well.
- 2c. Seal plate and incubate for 1 hour at 37°C in a humid atmosphere (e.g. on a water bath.

### 3. Enzyme coupling

- 3a. Wash the plate 3 times with wash buffer, as in step 2a.
- 3b. Add 200µl diluted peroxidase conjugate (1 in 100) per well.
- 3c. Seal plate and incubate for 1 hour at 37°C in a humid atmosphere (e.g. on a water bath).

### 4. Color reaction

- 4a. Wash the plate 3 times with wash buffer, as in step 2a.
- 4b. Add  $200\mu$ l freshly prepared TMB working solution (1 + 20 dilution) per well.
- 4c. Incubate for exactly 10 minutes at 20 27°C.
- 4d. Stop reaction by adding  $100\mu$ l STOP solution (1N H<sub>2</sub>SO<sub>4</sub>).
- 4e. Read absorbance within 10 minutes in a microplate reader at 450nm, with reference wavelength set to 620 650nm, if possible.

#### Suggested plate set-up:

The following plate arrangement may be chosen, where 300, 60, 12 and 0 are the standard dilutions in ng/ml. S1 through S44 are samples in duplicates.

	1	2	3	4	5	6	7	8	9	10	11	12
А	300	300	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
В	60	60	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
С	12	12	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	0	0	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
Е	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
Н	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

#### Calculation

Means are formed from duplicates and the content in the samples is calculated from the standard curve with the help of a microplate calculation software (e.g. Softmax, Molecular Device) or manually. The standard curve is best achieved with a linear extrapolation or by connecting the individual reference points. Sample dilutions which lie outside of the standard range may be measured a second time at a higher (lower) dilution, e.g. 1:500 (1:50).



Normal serum CD163 level in healthy donors is  $1.5\mu$ g/ml (range: 0.5 -  $3\mu$ g/ml, n=8). Intraassay variation has been determined <5% (n=12).

#### Limitations and incompatibilities:

Components from different lots or from different assays should never be mixed.

Normal values have been measured with serum of normal healthy donors. Corresponding EDTA or heparin plasma gives slightly different values.

It is recommended to use one sample dilution factor throughout all your experiments (e.g. 1:200) even though the dilution effect (going to 1:100 or 1:50) is minimal.

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