Macro163™

soluble CD163 ELISA assay
for the measurement of macrophage and monocyte activation

For Research Use Only. Not for Diagnostic Purposes.

IQ Products
bright fluorescence

Manual

REF IQP-383

96 tests

RUO For Research Use Only

IQ Products bv, The Netherlands

MACRO163™

soluble CD163 ELISA assay for the measurement of macrophage and monocyte activation

Intended use

This Macro163™ kit is intended for the quantification of soluble CD163 (sCD163) in serum or plasma samples. It is designed for research purposes only. The Macro163™ assay has been validated for serum and plasma measurements but can also be applied to other biological fluids like synovial fluid, ascites fluid, cerebrospinal fluid and cell-supernatants. In latter situations adjustments in sample dilutions need to be considered. The Macro163™ assay is completed within 3 hours with less than 1 hour of hands-on-time.

Background information

CD163 is a membrane protein and member of the group B Scavenger Receptor Cysteine-Rich super family specifically expressed on peripheral blood monocytes and macrophages. A particularly high expression is seen in macrophages of the 'alternative activation' phenotype playing a major role in dampening the inflammatory response and in scavenging components of damaged cells. CD163 functions as the receptor for Haptoglobin-hemoglobin complexes, and furthermore CD163 is involved in the regulation of inflammation. Macrophages play a central role in the host response to infection and tissue damage, and are furthermore important in the pathogenesis of autoimmune diseases and cancer. Historically, the presence of CD163 (by immune histochemistry) has been associated with a post-infectious recovery phase and declining inflammation. CD163 positive macrophages constitute a major cell subpopulation in human term placenta suggesting a role for the placenta functioning as an active immunosuppressive biological barrier between mother and fetus.
Importantly, the extracellular part of CD163 is cleaved from the cell-membrane by proteolytic activity upon toll-like receptor activation. This shed CD163 molecule (soluble CD163, sCD163) is detectable in body fluid compartments and is regarded to reflect activation and proliferation of macrophages in inflammatory conditions.
Measurement of sCD163 may be a valuable marker in diseases with macrophage and/or monocyte involvement, such as macrophage activation syndromes (e.g. hemophagocytic syndrome), infections, liver disease, auto-immune disease, atherosclerosis and cancer.

Principle of the Macro163™ assay

The assay is based on the principle of the sandwich ELISA. A polyclonal antibody recognizing CD163 is immobilized on the surface of a microtiterplate. After incubation with the sample or recombinant CD163 standard a second biotinylated monoclonal antibody recognizing CD163 is added. Detection of the latter is done by adding streptavidin-HRP. Using TMB (3, 3', 5, 5'-tetramethylbenzidine) as substrate for the enzyme HRP, the amount of sCD163 protein can be quantified.

Materials supplied with the Macro163™ kit

REF	Item	Description	Amount
A	Reagent A	Recombinant sCD163 standard. **Please check the label on the vial for the correct concentration**	2x vial
B	Reagent B	Biotinylated detection antibody. Contains 0.02% Sodium azide	135 µL
C	Reagent C	Streptavidine-HRP	150 µL
D	Reagent D (10x)	10x Washing buffer. Contains 0.01% Thimerosal.	40 mL
E	Reagent E (10x)	10x Dilution buffer for samples, standard and controls. Contains 0.01% Thimerosal.	10 mL
F	Reagent F 	TMB substrate. Contains <0.02% w/w TMB WARNING	11 mL
G	Reagent G 	Stop Solution (0.2 M H_2SO_4) WARNING	11 mL
H	Pre-coated microtiter plate	12x8 well pre-coated and pre-blocked strips	1x
I	Adhesive plate seals	Plate covers	4x
-	Manual	Instructions for use	1x

Laboratory material and equipment required, but not supplied with the Macro163™ kit

- Laboratory centrifuge for 1.5 mL vials
- 1.5 mL reaction tubes to prepare the recombinant CD163 standard and sample dilutions
- Distilled or demineralized water
- Adjustable micropipettes and tips
- Vortex mixer
- Stopwatch or timer
- Microtiterplate reader with 450 nm (and 620, 650, or 690 nm) filter and software to evaluate ELISA data

Storage

- Reagents stored according to stated storage instructions are stable until the expiration date indicated on the label. Store the reagents for repeated use immediately after usage at 2-8 °C.

Warning and precautions

- For Research Use Only.
- Reagents containing sodium azide (Reagent B [0,002%] may react with lead or copper plumbing to form explosive metal azides. On disposal, flush with large amounts of water to prevent azide build-up.
- All reagents should be handled in accordance with good laboratory practices using appropriate precautions.
- All samples should be handled with appropriate precautions.
- Do not pipette by mouth and wear gloves during the procedure.
- Reagents D and E contain Thimerosal (both 0.01%). Thimerosal may enter the body through the skin, is toxic, alters genetic material, May be irritating to the eyes and can cause allergic reactions. Avoid skin and eye contact and handle in accordance with good laboratory practices using appropriate precautions.
- Reagent F contains 3, 3', 5, 5'-tetramethylbenzidine (TMB; concentration <0.02%). The original manufacturer states that the preparation is not to be classified according to EEC directive 88/379/EEC. It is suggested to act in case of emergency as if the material should be. Avoid skin and eye contact and handle in accordance with good laboratory practices using appropriate precautions.

- Reagent G, the Stop Solution provided with this kit, is an acid solution. Protect face, eyes, hands and clothing by wearing appropriate protection when working with Reagent G (Stop Solution). Avoid skin and eye contact in accordance with good laboratory practices using appropriate precautions.
- The test must be performed by well-trained and authorized laboratory technicians.
- Please contact the manufacturer if the original test kit is damaged upon receipt.

Storage of the blood samples

- Blood should be collected in appropriate tubes. Only a few microliters are used in the Macro163™ assay.
- Serum, EDTA-, citrate- or heparin-anticoagulated plasma can be used for analyses.
- There is no interference of EDTA up to 10 mM or concentrations up to 200 mM of hemoglobin.
- Samples should be centrifuged (10 minutes at 1000 g) and serum/plasma drawn off by pipette within 24 hours.
- Soluble CD163 is stable in serum/plasma for more than 2 weeks at 4 °C, over 6 months at -20 °C, and for more than a year at -80 °C.
- After thawing samples can be re-frozen without loss of sCD163 reactivity (7).

Reagent preparation

- Before use, all buffers should be allowed to reach room-temperature (20-25 °C) except for reagent F (TMB) which is kept at 4 °C until section 6.
- Dilute the Washing buffer (reagent D) 10 times with demineralized water before use.
- Dilute the Dilution buffer (reagent E) 10 times with demineralized water before use.

For every Macro163™ 8-well pre-coated strip used we recommend to dilute:
- 0.5 ml reagent E (10x dilution buffer) with demineralized water to a volume of 5 ml,
- 3.5 ml reagent D (10x Washing buffer) with demineralized water to a volume of 35 ml.

Use of a complete Macro163™ pre-coated plate (12 strips) in one assay, we recommend to dilute:
- 6 ml reagent E (10x Dilution buffer) with demineralized water to a volume of 60 ml,
- 40 ml reagent D (10x Washing buffer) with demineralized water to a volume of 400 ml

- **Prior to the Macro163™ assay performance it is recommended to dilute the samples. As the samples may derive from various sources, the following dilutions in 1x Dilution buffer are recommended:**
  - Serum/plasma 1:500
  - Synovial fluid 1:2500
  - Ascites, pleural or pericardial fluid 1:500
  - Cerebrospinal fluid 1:50

Macro163™ Assay Procedure

The procedure roughly consists of the following steps

- CD163 standard preparation step 1
- Sample preparation step 2
- Sample and Standard incubation step 3
- Detection-antibody incubation step 5
- Streptavidine-HRP incubation step 7
- TMB substrate reaction step 9
- Adding Stop Solution step 10
- Data analysis

Preparations prior to Macro163™ Assay Procedure

- The incubation steps as described in this section may be performed on a microplate shaker under gentle shaking. This is not necessary for a successful assay performance.
- Prior to their use *reagents A, B and C should be spinned down* for 30 seconds at full speed before opening.

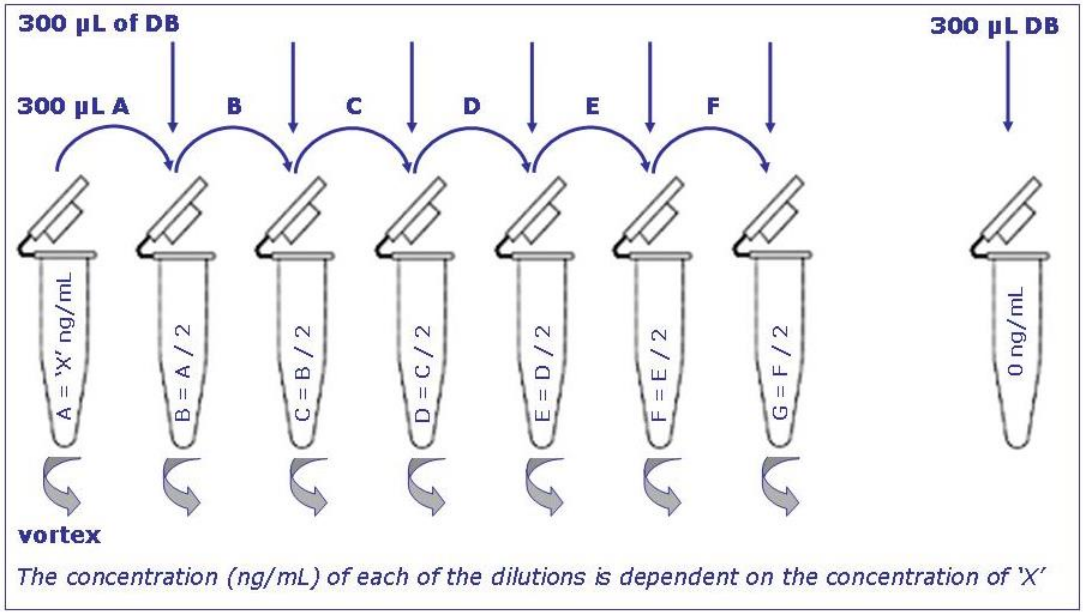
Assay Procedure

1- Preparation of the recombinant sCD163 standard

- Shortly spin the reagent A vial (recombinant sCD163 standard) to make sure all material is at the bottom of the vial before opening. One vial is enough to prepare one standard curve in duplo.
- Prepare reagent A by dissolving the material in 600 µl 1x reagent E (= dilution buffer; referred to as '**DB**' in the illustration below). Close the vial and mix thoroughly after addition of the buffer and let rest for 15 minutes.
- **To use the correct concentration of the sCD163 standard in the preparation of the standard dilution curve, check the label on the vial. The concentration after addition of 1x reagent E is stated on the label of the vial.**
- The starting concentration or first point in the standard curve, is the one that is mentioned on the vial (in the illustration noted as **'X'**).
- From here, the standard dilution curve is a repeated sequence a 2 fold serial dilution described as B – F in the illustration below. 300 µL of the preceding dilution is added to 300 µL of DB. Vortex after mixing.

Note

- For the measurement of samples with very low concentrations of sCD163 (e.g. cerebrospinal fluids or cell culture media) it is advised to discard the value of the highest point standard curve. Instead; make the same dilution curve, but dilute the standard one more step. Start the curve at the second point .



The concentration (ng/mL) of each of the dilutions is dependent on the concentration of 'X'

Sample preparation

2– Sample Preparation

- Dilution can be performed by using 1.5 mL reaction tubes.
- Duplicate measurements are recommended.
- Prepare a 1:50 dilution of the serum or plasma sample to be tested by adding 10 uL of sample to 490 uL of Reagent E Dilution buffer. Mix gently by vortexing.
- Prepare a 1:500 final dilution of the sample by adding 50 uL of the 1:50 dilution prepared in step to 450 uL of Reagent E Dilution buffer. Mix gently by vortexing. The volume of the 1:500 sample is sufficient for 4 tests for each sample.
- (This dilution strategy is recommended for serum and plasma samples, resulting in a 1:500 final dilution. Final dilutions can be adjusted to achieve the recommended final dilution for other sample types as described in the table in the Reagent Preparation section.)

3– Adding standards and samples to the pre-coated wells and incubation of samples and standards

- Add 100 uL of each standard dilution (Reagent A, prepared previously), or sample dilution (prepared in step 2) in duplicate to each well.
- Add 100 uL of reagent E to two blank wells.
- Mix the fluids gently by tapping the side of the plate or by using a microplate shaker under gentle shaking.
- Seal the plate.
- Incubate at room temperature (20-25C) for 1 hour.
- During this incubation, the detection antibody (reagent B) is prepared (next step).

Preparation of the Detection-antibody (reagent B)

- Dilute the 100x reagent B (100 x detection antibody) in 1x reagent E. For example: Add 100 µL of 100x reagent B to 10 ml of 1x reagent E. This is sufficient for a full microtiter plate.

4- Washing (manual)

- After the 1 hour incubation the samples/standards are aspirated.
- Invert the plate on a lint-free tissue and tap dry.
- Dispense 200 - 300 µL 1x reagent D into each well, than aspirate the washing solution.
- Invert plate on a tissue and tap dry.
- Repeat this step for 2 more times.

5- Detection-antibody incubation

- Add 100 µL of the 1x detection antibody (reagent B) to each well using a multi-channel pipette.
- Seal the plate with the plate sealer.
- Incubate at room temperature (20-25 °C) for 1 hour.
- During this incubation the streptavidin-HRP solution (reagent C) is prepared (next step).

Preparation of the streptavidine-HRP solution (reagent C)

- Dilute 100x reagent C in 1x reagent E. For example: Add 100 µL of reagent C to 10 mL 1x reagent E. This is sufficient for a full microtiter plate.

6- Washing (manual)

- After the 1 hour incubation the diluted reagent B solution is aspirated.
- Invert the plate on a lint-free tissue and tap dry.
- Dispense 200 - 300 µL 1x reagent D into each well, than aspirate the 1x reagent D.
- Invert plate on a tissue and tap dry.
- Repeat this step for 2 more times.

7- Streptavidine-HRP incubation

- Add 100 µL 1x streptavidine-HRP solution (reagent C) to each well using a multi-channel pipette.
- Seal the plate with the plate sealer.
- Incubate at room temperature (20-25 °C) for 1 hour.
- Aliquot the needed volume of reagent F (number of wells (100 µl/well) plus an extra 20% of volume) from the stock bottle reagent F with a clean pipette and close the bottle firmly. Wrap it in aluminum foil and place it in the dark. Reagent F is light sensitive! Place the stock bottle of reagent F back at 2-8 °C.
- Allow the aliquot of TMB to reach room temperature (20-25 °C).

8- Washing (manual)

- After the 1 hour incubation the Streptavidine-HRP solution is aspirated.
- Invert the plate on a lint-free tissue and tap dry.
- Dispense 200 - 300 µL 1x reagent D into each well, than aspirate the 1x reagent D.
- Invert plate on a tissue and tap dry.
- Repeat this step for 4 more times.

9- Adding the TMB

- Add 100 µL of TMB (reagent F) to each well using a multi-channel pipette.
- Seal the plate with a *new* plate sealer.
- Incubate at room temperature (20-25 °C) for 15 min in the dark.

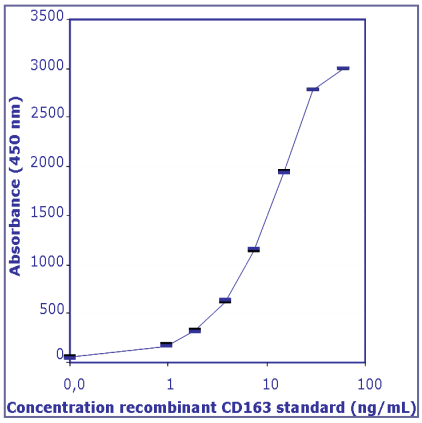
10- Adding the Stop solution

- Add 100 µL Reagent G (Stop Solution) to each well using a multi-channel pipette.
- Mix the fluids gently by tapping the side of the plate or by using a microplate shaker under gentle shaking.
- Read the plate with a 450 nm filter within 15 minutes of adding 0.2 M H_2SO_4 (a 620, 650, or 690 nm filter can be used as reference wavelength).

Data analysis

i. Standard curve

The standard curve can be generated by linear interpolation or by automated curve fitting methods as supplied by the standard ELISA software programs. A typical example of a sCD163 ELISA standard curve is shown to the right



ii. Traceability/correctness

The assay correlates well (r^2 = 0.97) with published assays used for clinical studies. The assay was calibrated against a purified human CD163 standard.

iii. Precision of the Macro163™ assay

Intra-assay variability is 3-6 %; Inter-assay variability is 5-8 % when measuring in duplicates.

iv. Linearity of the Macro163™ assay

It was shown to be linear from 0.9 – 30 ng/mL.

v. Detection level of the Macro163™ assay

Less than 0.23 ng/mL.

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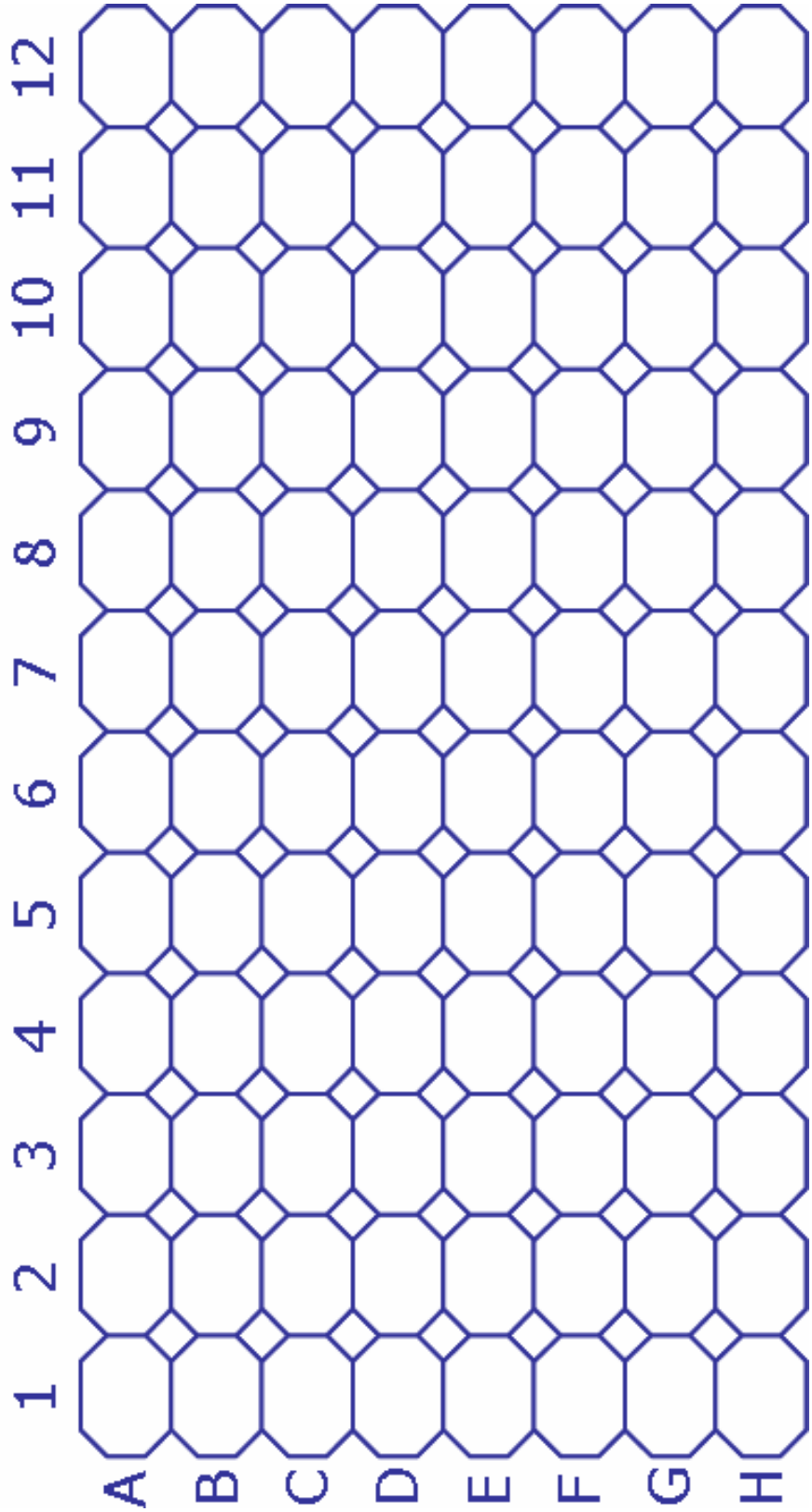
Sampling scheme



Warranty

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products bv is not liable for property damage, personal injury, or economic loss caused by the product.

Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer

Contact information

IQ Products BV
www.iqproducts.nl

Rozenburglaan 13a
9727 DL Groningen
The Netherlands
T 31 (0)50 5757000
F 31 (0)50 5757002
marketing@iqproducts.nl