Quantikine® ELISA

Human CD163 Immunoassay

Catalog Number DC1630

For the quantitative determination of human CD163 concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

CD163, previously called M130 or p155, is a 130 kDa type I transmembrane protein belonging to group B of the cysteine-rich scavenger receptor family (1-3). It is essential for clearance of hemoglobin-haptoglobin (Hb-Hp) complexes in the liver, spleen, and in circulation (4). Expression of CD163 is constitutive and/or induced by glucocorticoids, IL-10, IL-6, or endotoxin, on circulating monocytes, most tissue macrophages, and at low levels on monocyte-derived dendritic cells (1, 2, 5, 6). It is not present on macrophages in the mantle zone and germinal centers of lymphoid follicles, interdigitating reticulum cells, or Langerhans cells (6). The 1009 amino acid (aa) extracellular domain of CD163 contains 9 scavenger receptor cysteine-rich (SRCR) domains (1). The third SRCR domain is crucial for calcium-dependent binding of hemoglobin/haptoglobin complexes (3). Four isoforms vary in both intracellular and extracellular regions (1, 7). The C-terminal 42 aa of the 84 aa cytoplasmic domain are substituted with 48 alternate aa in isoform 2 and by 6 alternate aa in isoforms 3 and 4. Isoform 4 also has a 34 aa insert between SRCR domains 5 and 6. While all are expressed, isoform 3 is most abundant, most expressed on the cell surface, and most active in endocytosis (7). An approximately 130 kDa soluble form of human CD163 (sCD163) is assumed to contain virtually all of the extracellular domain. It shares approximately 75% aa sequence identity with mouse and rat sCD163 (8, 9). It is released from the cell surface by proteolysis after oxidative stress or inflammatory stimuli, including bacterial endotoxins and activation of the Toll-like receptors TLR2 or TLR5 (9-12).

Circulating free hemoglobin (Hb) is highly toxic due to the redox-reactive iron it contains. Iron is also a limiting nutrient for pathogen growth (11). When present in the circulation, free Hb is quickly complexed with haptoglobin (Hp). The complexes are then cleared by CD163, which is their only known receptor (4). Of haptoglobin isotypes, the 2-2 dimer has higher affinity for CD163 than the 1-1 dimer (4). CD163 will also take up free Hb at low affinity if Hp is depleted or overwhelmed (13). CD163-mediated uptake of Hb-Hp induces a signaling cascade resulting in upregulation of heme oxygenase-1 (HO-1), a critical enzyme for recycling of heme iron (14, 15). Soluble CD163 (sCD163) also has a protective and anti-inflammatory role, as shown by its ability to inhibit phorbol ester-induced lymphocyte proliferation (16). In addition to Hb-Hp, monocyte CD163 can bind the TNF-like weak inducer of apoptosis (TWEAK), which may serve to sequester TWEAK from its primary receptor, Fn14/TWEAK R (17). The second SRCR of CD163 on bone marrow macrophages has an additional function, acting as an erythroblast adhesion receptor that enhances erythropoiesis (18). Some inflammatory conditions are associated with disregulation of CD163 or of its shedding (19-22). Decreased production of cellular CD163 within atherosclerotic plaques and increased circulating sCD163 may occur in diabetes mellitus (19). Elevated sCD163 has also been reported in multiple sclerosis and liver failure, and in the synovial fluid in spondyloarthropathy synovitis but not rheumatoid arthritis (20-22).

The Quantikine® Human CD163 immunoassay is a 4.5 hour solid phase ELISA designed to measure human soluble CD163 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human CD163 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CD163 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for human CD163.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CD163 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD163 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human CD163 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CD163 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human CD163 Microplate	893269	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human CD163.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human CD163 Standard	893271	Recombinant human CD163 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human CD163 Conjugate	893270	21 mL of a monoclonal antibody specific for human CD163 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-34	895265	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human CD163 Controls (optional; R&D Systems®, Catalog # QC61).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA plasma is not suitable for use in this assay due to its chelating properties.*
Citrate plasma has not been validated for use in this assay.
Hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-24 (diluted 1:2)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

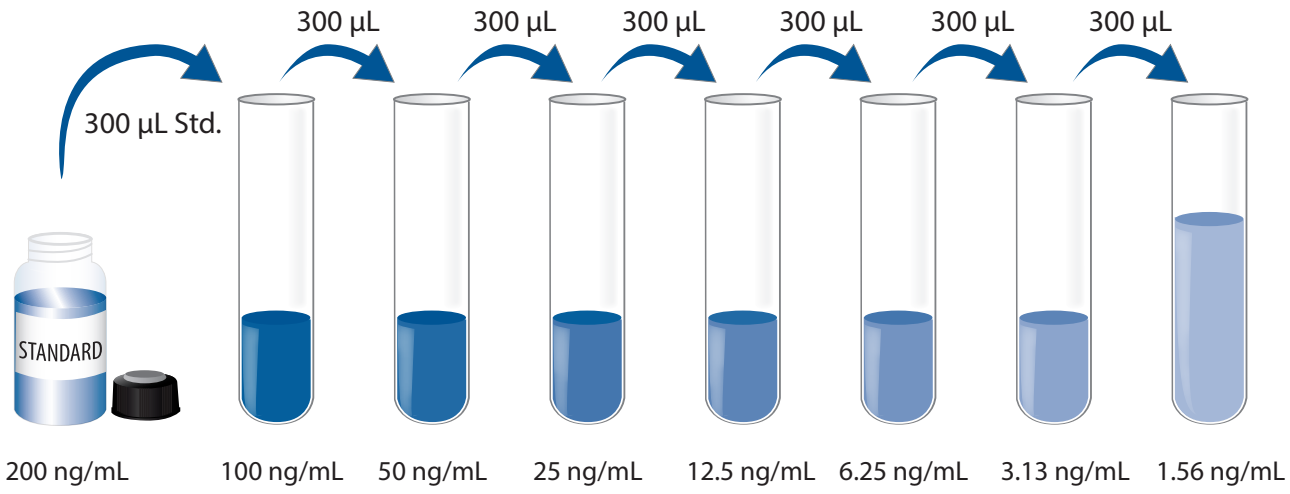
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-24 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5-24 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-24 (diluted 1:2).

Human CD163 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CD163 Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD5-24 (diluted 1:2) into the 100 ng/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 100 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (diluted 1:2) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-34 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human CD163 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

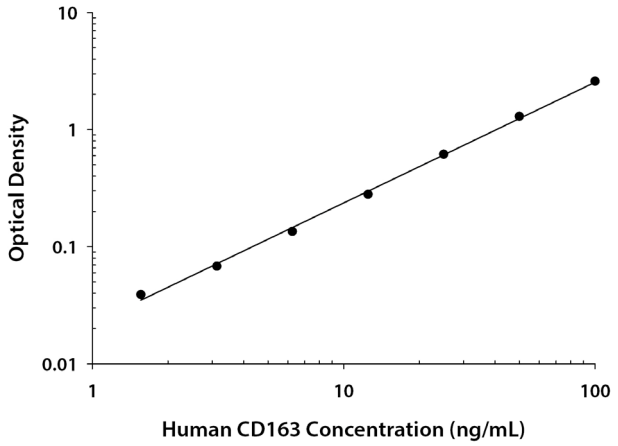
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human CD163 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.011	0.011	—
1.56	0.039 0.039	0.039	0.028
3.13	0.066 0.069	0.068	0.057
6.25	0.134 0.135	0.135	0.124
12.5	0.276 0.284	0.280	0.269
25	0.608 0.622	0.615	0.604
50	1.261 1.330	1.296	1.285
100	2.585 2.587	2.586	2.575

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	20.0	35.1	65.6	20.0	34.9	63.6
Standard deviation	0.75	1.2	2.3	1.3	1.6	2.6
CV (%)	3.8	3.4	3.5	6.5	4.6	4.1

RECOVERY

The recovery of human CD163 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	107	101-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CD163 were serially diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	102	109	109
	Range (%)	98-105	105-113	105-116
1:4	Average % of Expected	100	104	101
	Range (%)	98-103	100-109	94-111
1:8	Average % of Expected	100	95	96
	Range (%)	96-103	90-100	92-104
1:16	Average % of Expected	97	88	90
	Range (%)	93-101	87-89	84-104

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Forty-six assays were evaluated and the minimum detectable dose (MDD) of human CD163 ranged from 0.058-0.613 ng/mL. The mean MDD was 0.177 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human CD163 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CD163 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	472	88-902	186
Heparin plasma (n=36)	521	186-996	203

Cell Culture Supernates:

PBLs human peripheral blood lymphocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL of PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human CD163.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	ND	5.0
Stimulated	ND	4.4

ND=Non detectable

Human monocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin sulfate, 10 ng/mL of recombinant human IFN-γ, 7.0 ng/mL of recombinant human IL-10, and 5.0 µg/mL of lipopolysaccharide (LPS). An aliquot of the cell culture supernate was removed, assayed for human CD163, and measured 64.9 ng/mL.

Human monocytes were cultured in RPMI supplemented with 10% fetal bovine serum and 25 ng/mL of recombinant human GM-CSF. In the last 24 hours, they were stimulated with 50 ng/mL of LPS. An aliquot of the cell culture supernate was removed, assayed for human CD163, and measured 20.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human CD163.

The factors listed below were prepared at 1000 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 1000 ng/mL in a mid-range recombinant human CD163 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
CD5
CD6
DMBT1
TWEAK

Recombinant mouse:
CD5
CD6
TWEAK

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