Human CD163 Sandwich ELISA Kit Datasheet

For the quantitative detection of human CD163 concentrations in serum and plasma.

General Information

Catalogue Number	KE00190
Product Name	Human CD163 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	6.25 - 400 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	9332
SwissProt	Q86VB7

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 400 ng/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 3-ec - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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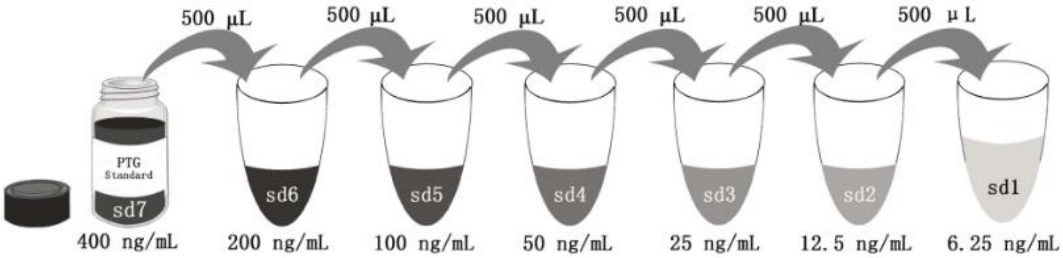
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-ec is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **1mL** Sample Diluent PT 3-ec in protein standard. This reconstitution gives a stock solution of 400 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent **PT 3-ec**	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00190 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CD163 ELISA kit is to be used to detect and quantify protein levels of endogenous CD163. The assay recognizes human CD163. An antibody specific for CD163 has been pre-coated onto the microwells. The CD163 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for human CD163 is added to detect the captured human CD163 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

CD163, also known as M130, is a membrane glycoprotein which belongs to the scavenger receptor superfamily. It is an acute phase-regulated and signal-inducing macrophage protein expressed exclusively in monocytes and tissue macrophages. CD163 mediates endocytosis of haptoglobin-haemoglobin complexes. The uptake of haptoglobin by macrophages contributes to the recycling of iron and also to the inflammatory response. Soluble CD163 (sCD163), as a result of ectodomain shedding during inflammatory activation of macrophages, circulates in blood and has been suggested as a plasma/serum marker for macrophage activity.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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Safety Notes

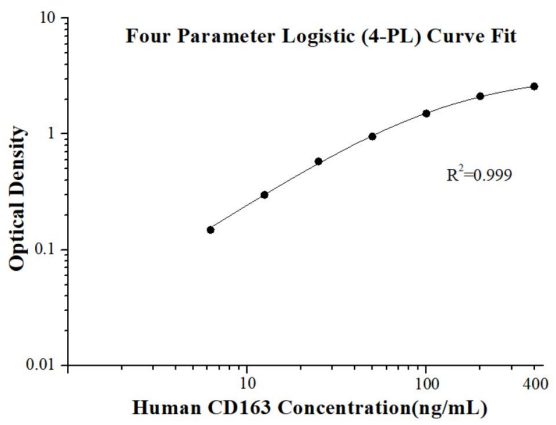
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.057 0.058	0.058	-
6.25	0.214 0.198	0.206	0.149
12.5	0.371 0.340	0.356	0.298
25	0.614 0.661	0.638	0.580
50	1.013 1.007	1.010	0.953
100	1.588 1.535	1.562	1.504
200	2.168 2.185	2.177	2.119
400	2.589 2.675	2.632	2.575

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	44.3	1.2	2.8
2	20	64.2	1.7	2.7
3	20	195.9	6.7	3.4

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	50.9	3.7	7.2
2	24	76.4	6.2	8.1
3	24	247.5	21.3	8.6

Recovery

The recovery of CD163 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:4	90	80-107
	1:8	97	78-112

Sample Values

Serum and plasma samples from healthy volunteers were evaluated for CD163 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human plasma (n=32)	233.2	71.1-642.9
Human serum (n=24)	294.2	100.7-789.3

Sensitivity

The minimum detectable dose of human CD163 is 1.2 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, Serum were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	100	-
	1:4	114	105-123
	1:8	97	88-106
	1:16	76	74-78

References

1. Law SK, Micklem KJ, Shaw JM, et al. A new macrophage differentiation antigen which is a member of the scavenger receptor superfamily. *Eur J Immunol.* 1993;23(9):2320-2325.
2. Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger receptor. *Nature.* 2001;409(6817):198-201.
3. Etzerodt A, Moestrup SK. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxid Redox Signal.* 2013;18(17):2352-2363.
4. Møller HJ, Aerts H, Grønbaek H, et al. Soluble CD163: a marker molecule for monocyte/macrophage activity in disease. *Scand J Clin Lab Invest Suppl.* 2002;237:29-33.

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