PRODUCT INFORMATION & MANUAL

Human CD163 Valukine™ ELISA

Catalog Number: VAL205

For the quantitative determination of natural and recombinant human CD163 concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202410.1

TABLE OF CONTENTS

I. BACKGROUND

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).

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II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

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

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate, human serum and human plasma.
- 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, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in forty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
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

B. RECOVERY

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

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

C. 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 optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

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

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human CD163 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Human Serum* (n=4)	Human 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.

F. SAMPLE VALUES

Human 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)
Human Serum (n=36)	472	88-902	186
Human Heparin plasma (n=36)	521	186-996	203

Cell Culture Supernates:

PBLs human peripheral blood lymphocytes (1 × 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.

G. SPECIFICITY

This assay recognizes natural and recombinant human CD163.

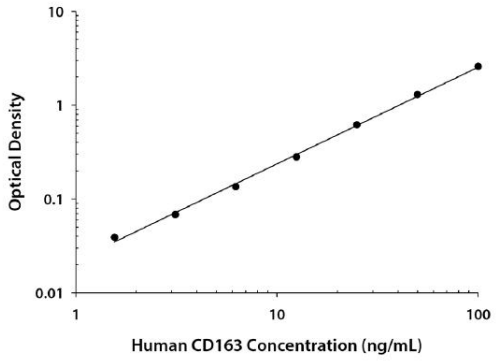
The factors listed below were prepared at 1000 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 1000 ng/mL in a mid-range recombinant human CD163 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：	Recombinant mouse:
CD5	CD5
CD6	CD6
DMBT1	TWEAK
TWEAK	

IV. EXPERIMENT

EXAMPLE STANDARD

The 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

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human CD163 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human CD163	1 plate
Human CD163 Conjugate	Solution of antibody against human CD163 conjugated to horseradish peroxidase	1 vial
Human CD163 Standard	Recombinant human CD163 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-34	A buffered protein base	1 vial
Calibrator Diluent Concentrate (2×)/ RD5-24	A 2× concentrated buffered protein base used to dilute standard and samples	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Assay Diluent RD1-34</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent Concentrate (2×)/ RD5-24</td><td>May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. 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.
- 100 mL and 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND 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. Samples may require dilution with Calibrator Diluent (1×).

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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

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.

B. SAMPLE PREPARATION

Human serum and human plasma samples recommend a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

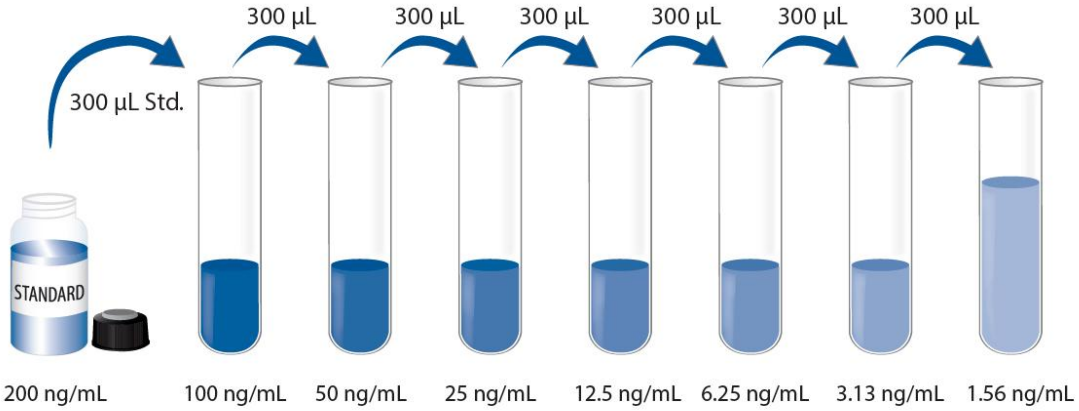
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

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

*If you have any question, please seek help from our Technical Support.

Pipette 300 µL of Calibrator Diluent (1×) 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 (1×) serves as the zero standard (0 ng/mL).



D. 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

VII.ASSAY PROCEDURE

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

1. Prepare all reagents and working standards 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 and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
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 TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 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.
11. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard 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.

VIII. REFERENCES

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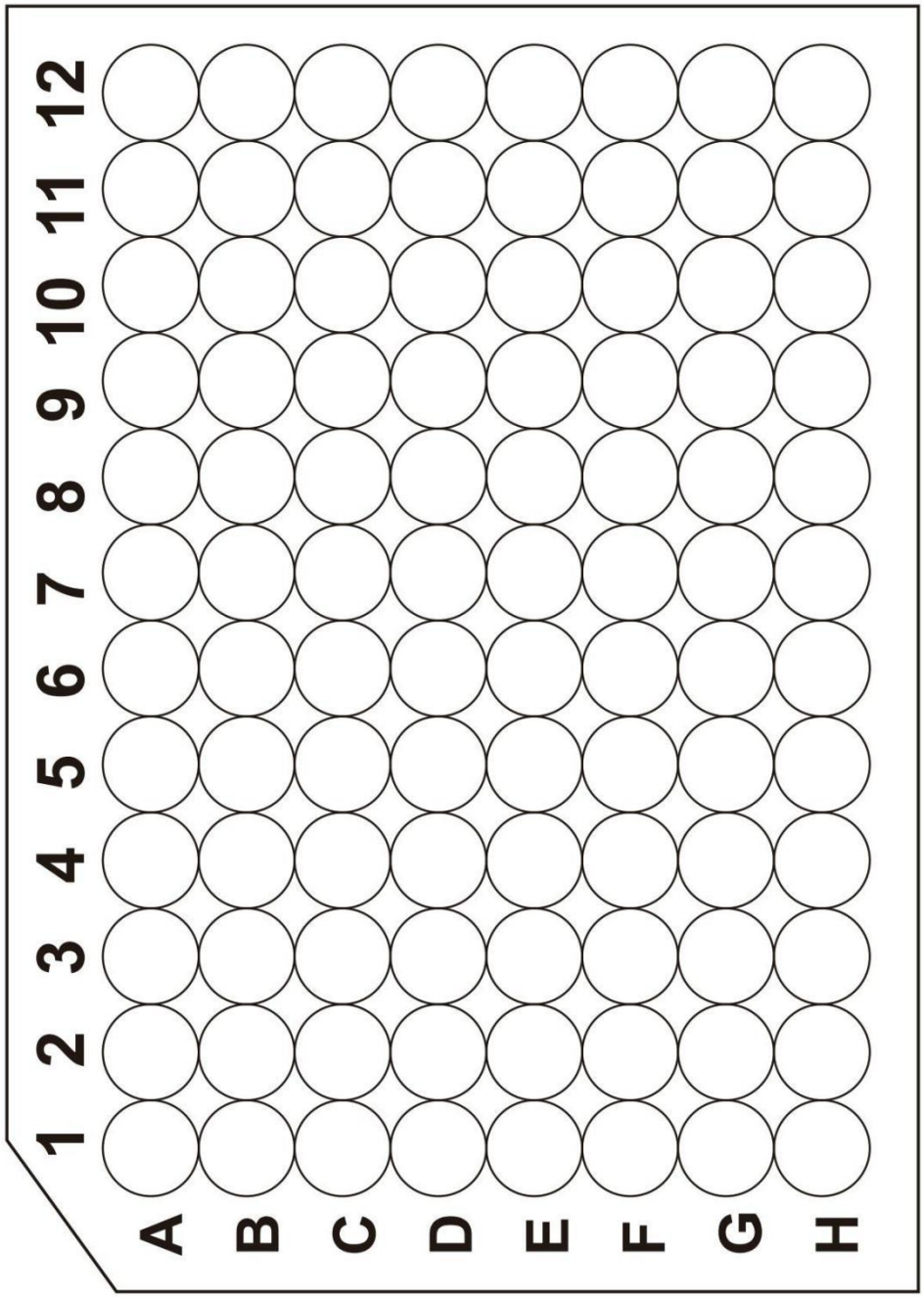
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 CD163 Valukine™ ELISA 试剂盒

目录号：VAL205

适用于定量检测天然和重组人 CD163 的浓度

科研专用，不可用于临床诊断

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Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202410.1

目录

I. 背景

CD163 以前称为 M130 或 p155，是一种 130 kDa 的 I 型跨膜蛋白，属于富含半胱氨酸的清道夫受体家族 B 组(1-3)。它对于血红蛋白-触珠蛋白复合物（Hb-Hp）在肝脏、脾脏和循环中的清除至关重要(4)。CD163 的表达和/或诱导受多种因素调节，如糖皮质激素、IL-10、IL-6 或内毒素，在循环单核细胞、大多数组织巨噬细胞以及单核细胞衍生的树突状细胞上以低水平表达(1, 2, 5, 6)。它不存在于淋巴滤泡的外套区和生发中心的巨噬细胞、叉间网状细胞或朗格汉斯细胞上 (6)。CD163 的 1009 个氨基酸（aa）胞外结构域包含 9 个富含半胱氨酸的清道夫受体（SRCR）结构域(1)。其中第三个 SRCR 结构域对于血红蛋白-触珠蛋白复合物的钙依赖性结合至关重要(3)。四种亚型在胞内和胞外区域均不同(1, 7)。在异构体 2 中，84 个氨基酸的细胞质结构域中 C 端的 42 个氨基酸可被 48 个氨基酸取代，在异构体 3 和 4 中有 6 个氨基酸被取代。异构体 4 在 SRCR 结构域 5 和 6 之间也有一个 34aa 的插入。虽然所有异构体都能被表达，但异构体 3 的表达量最大，在细胞表面的表达最多，而且在内吞过程中最为活跃 (7)。人 CD163 的可溶形式（sCD163）约为 130 kDa，据推测它几乎包含了所有的细胞外结构域。小鼠和大鼠 sCD163 大约有 75%的氨基酸序列相同 (8, 9)。在受到氧化压力或炎症刺激（包括细菌内毒素和 Toll 样受体 TLR2 或 TLR5 的激活）后，它会通过蛋白水解作用从细胞表面释放出来(9-12)。

循环游离血红蛋白（Hb）含有氧化还原反应铁，因此毒性很强。铁也是病原体生长的一种限制性营养物质(11)。游离 Hb 进入血液循环后，会迅速与 Hp 形成复合物(4)。然后，这些复合物会被 CD163 清除，CD163 是它们唯一已知的受体(4)。在血红蛋白异型中，2-2 二聚体对 CD163 的亲和力高于 1-1 二聚体(4)。如果 Hp 消耗殆尽，则 CD163 对游离 Hb 具有较低的亲和力。CD163 介导的对 Hb-Hp 的吸收会诱导一个信号级联，导致 HO-1 的上调，HO-1 是回收血红素铁的一种关键酶(14, 15)。可溶性 CD163（sCD163）还具有保护和抗炎作用，其表现为它还能抑制光稳定剂诱导的淋巴细胞增殖(16)。除 Hb-Hp 外，单核细胞 CD163 还能与 TWEAK 结合，从而将 TWEAK 从其主要受体 Fn14/TWEAK R 中分离出来(17)。骨髓巨噬细胞上 CD163 的第二个 SRCR 还有一个额外功能，即充当红细胞粘附受体，促进红细胞生成(18)。某些炎症情况与 CD163 的失调或脱落有关(19-22)，如糖尿病患者动脉粥样硬化斑块中细胞 CD163 的生成减少，而循环中的 sCD163 增加(19)。多发性硬化症和肝功能衰竭以及脊柱关节病滑膜炎的滑液中也有 sCD163 升高的报道，但类风湿性关节炎没有 (20-22)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人CD163抗体包被于微孔板上，样品和标准品中的人CD163会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人CD163检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本，人血清和人血浆；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测40次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (ng/mL)	20.0	35.1	65.6	20.0	34.9	63.6
标准差	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

B. 回收率

不同类型样本中掺入检测范围内不同水平的人CD163，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	107	101-110

C. 灵敏度

进行46次检测评估，人CD163的最低可检测剂量（MDD）范围为0.058-0.613 ng/mL。平均MDD为0.177 ng/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组人CD163校正。

E. 线性

不同的样本中含有或掺入高浓度的人CD163，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基 (n=4)	人血清* (n=4)	人肝素血浆* (n=4)
1:2	平均值/期待值（%）	102	109	109
	范围 (%)	98-105	105-113	105-116
1:4	平均值/期待值（%）	100	104	101
	范围 (%)	98-103	100-109	94-111
1:8	平均值/期待值（%）	100	95	96
	范围 (%)	96-103	90-100	92-104
1:16	平均值/期待值（%）	97	88	90
	范围 (%)	93-101	87-89	84-104

*按照样品制备部分的指示，在测定前稀释样品。

F. 样本预值

人血清/血浆–在本试验中评估了来自表面健康志愿者的样本中是否存在人 CD163。本研究中使用的供体没有病史。

样本类型	平均值 (ng/mL)	范围 (ng/mL)	标准偏差（ng/mL）
人血清 (n=36)	472	88-902	186
人肝素血浆 (n=36)	521	186-996	203

细胞培养上清：

PBLs（人外周血淋巴细胞，1 × 10^6 cells/mL）在含5% 胎牛血清、5 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL 链霉素硫酸盐的DMEM中培养。细胞在未刺激或用10 μg/mL PHA刺激后培养。等分细胞培养上清液，并测定人CD163的水平。

条件	1 天 (ng/mL)	6 天 (ng/mL)
未刺激	ND	5.0
刺激	ND	4.4

ND=未检出

人单核细胞在RPMI培养基中培养，该培养基补充了10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素、100 μg/mL链霉素硫酸盐、10 ng/mL重组人IFN-γ、7.0 ng/mL重组人

IL-10和5.0 μg/mL脂多糖（LPS）。等分细胞培养上清液，并测定人CD163，测得浓度为 64.9 ng/mL。

人单核细胞在补充了10%胎牛血清和25 ng/mL重组人GM-CSF的RPMI培养基中培养。最后24小时，用50 ng/mL的LPS刺激。等分细胞培养上清液，并测定人CD163，测得浓度为20.3 ng/mL。

G. 特异性

检测方法识别天然和重组人CD163。

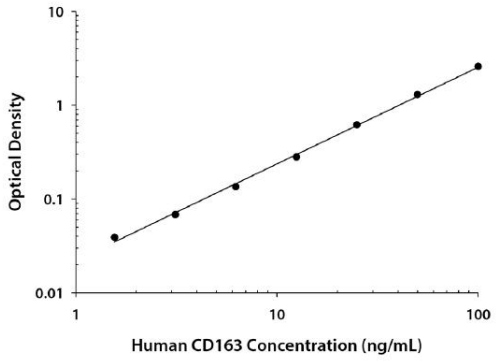
以下列出的因子在标准品稀释液（1×）中以1000 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人CD163对照品中以1000 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human：	**Recombinant mouse:**
CD5	CD5
CD6	CD6
DMBT1	TWEAK
TWEAK	

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



(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

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human CD163 Microplate	包被抗人CD163抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human CD163 Conjugate	酶标检测抗人CD163抗体	1瓶
Human CD163 Standard	重组人CD163标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-34	检测液	1瓶
Calibrator Diluent Concentrate (2×)/ RD5-24	浓缩的标准品稀释液（2×），用于稀释标准品和样品	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>分装并储存在 ≤ -20 °C 的冰箱中*，避免反复冻融循环</td></tr>
<tr><td>检测液RD1-34</td><td>2-8℃储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（2×）/ RD5-24</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8 ℃储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管（SST），让样本在室温下凝固 30 分钟，然后在 1000 × g 的离心力下离心 15 分钟。分离血清并立即进行检测，或分装并储存在 ≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液（1×）进行稀释。

血浆 - 使用肝素作为抗凝剂收集血浆。在采样后 30 分钟内，以 1000 × g 的离心力离心 15 分钟。立即检测或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

注：EDTA血浆由于其螯合特性，不适合用于该检测。

柠檬酸盐血浆在本检测中未经验证。

溶血样品不适合用于该检测。

B. 样品准备

人血清和人血浆样品建议稀释10倍。建议的10倍稀释度为20 µL样品+180 µL标准品稀释液 (1×)。最佳稀释倍数应由用户确定。

C. 检测前准备工作

注意：*使用前请将所有试剂放置于室温。*

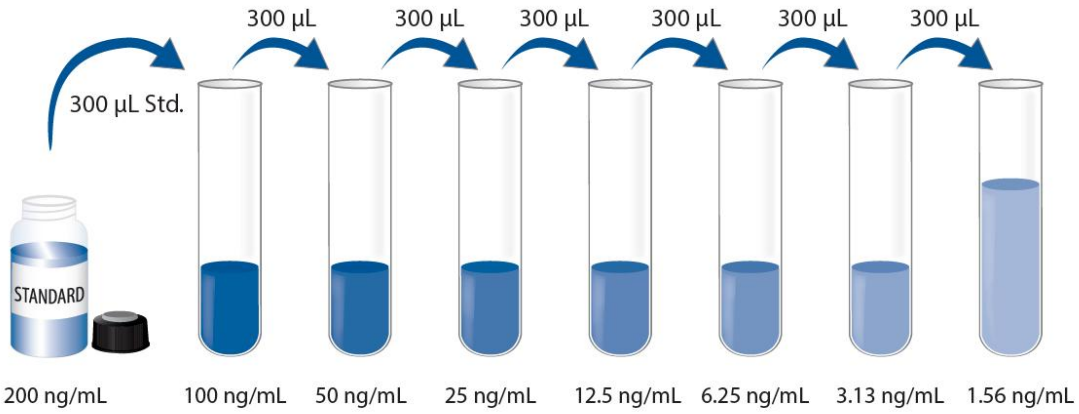
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人CD163标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人CD163标准品，得到浓度为200 ng/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将300 µL标准品稀释液（1×）移入100 ng/mL管中。用移液管吸取300 µL至其余管中。使用储备母液溶液稀释（如下）。在下次转移之前，将每根管彻底混合。100 ng/mL作为最高标准点。标准品稀释液（1×）作为标准品零点（0 ng/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄； 若孔内有绿色，则表明孔内液体未混匀请充分混合

VII. 操作步骤

使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 向每个孔中加入100 µL检测液RD1-34。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**在室温下孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入200 µL人CD163酶标检测抗体。用封板膜封住反应孔，**在室温下孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**；
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作log/log曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人CD163浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置