Datasheet for ABIN1380017

CD163 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CD163
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	157-10000 pg/mL
Minimum Detection Limit:	157 pg/mL
Application:	ELISA

Product Details

Purpose:	The OmniKine? Human CD163 ELISA Kit contains the components necessary for quantitative determination of natural or recombinant Human CD163 concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a "Sandwich" Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a "sandwich" format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on Human CD163 while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and "sandwiching" of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'-Tetramethylbenzidine) is

	added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.
Brand:	OmniKine™
Sample Type:	Cell Lysate, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The Human CD163 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human CD163 proteins.
Cross-Reactivity (Details):	The Human CD163 ELISA is capable of recognizing both recombinant and naturally produced Human CD163 proteins. The antigens listed below were tested at 100 ng/mL and did not exhibit significant cross-reactivity or interference. Human: CF6, TWEAK Murine: CF6, CD163, TWEAK
Characteristics:	The Human CD163 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human CD163 proteins within the range of 157-10000 pg/mL.
Components:	• Microstrips Coated w / Capture Antibody: 12 x 8-Well Microstrips • Protein Standard: Lyophilized (100 ng), Red container • Biotinylated Detection Antibody: Lyophilized, Yellow container • 400x Streptavidin-HRP: 30 µL, Blue container • Wash Buffer (10x): 50 mL, Clear containter • Assay Diluent: 50 mL, Clear container • Ready-to-Use Substrate: 12 mL, Brown container • Stop Solution: 12 mL, Clear container • Adhesive Plate Sealers: 4 Sheets • Technical Manual 1 Manual
Material not included:	The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm) Micropipettes with capability of measuring volumes ranging from 1 µl to 1 mL Deionized or sterile water Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer

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	Graph paper or computer software capable of generating or displaying logarithmic functions
	Absorbent paper or vacuum aspirator
	Test tubes or microfuge tubes capable of storing ≥1 mL
	Bench
	top centrifuge (optional)
	Bench
	top vortex (optional)
	Orbital shaker (optional)

Target Details

Target:	CD163
Alternative Name:	CD163 (CD163 Products)
Background:	CD163, also known as Scavenger Receptor Cysteine-rich Type 1 Protein M130, is an 1156 amino acid secreted protein that is found on cell membranes. It is a single-pass type I membrane protein with isoform 1 and isoform 2 showing lower surface expression when expressed in cells. CD163 interacts with CSNK2B and is expressed in monocytes and mature macrophages such as Kupffer cells in the liver, red pulp macrophages in the spleen, cortical macrophages in the thymus, resident bone marrow macrophages and meningeal macrophages of the central nervous system. It is also expressed in blood. Isoform 1 is the lowest abundant in the blood. Isoform 2 is the lowest abundant in the liver and the spleen. Isoform 3 is the predominant isoform detected in the blood. CD 163 is induced by anti- inflammatory mediators such as glucocorticoids, interleukin-6/IL6 and interleukin-10/IL10, it is suppressed by proinflammatory mediators like bacterial LPS, IFN-gamma and TNF. CD163 functions as an acute phase- regulated receptor involved in clearance and endocytosis of hemoglobin/haptoglobin complexes by macrophages and it may thereby protect tissues from free hemoglobin-mediated oxidative damage. CD163 binds hemoglobin/haptoglobin complexes in a calcium-dependent and pH- dependent manner, it exhibits a higher affinity for complexes of hemoglobin and multimeric haptoglobin of HP*1F phenotype than for complexes of hemoglobin and dimeric haptoglobin of HP*1S phenotype. CD163 induces a cascade of intracellular signals that involves tyrosine kinase-dependent calcium mobilization, inositol triphosphate production and secretion of IL6 and CSF1. Isoform 3 exhibits the higher capacity for ligand endocytosis and the more pronounced surface expression when expressed in cells. The SRCR domain 3 mediates calcium-sensitive interaction with hemoglobin/haptoglobin complexes. A soluble form (sCD163) of CD163 is produced by proteolytic shedding which can be induced by lipopolysaccharide, phorbol ester and Fc region of immunoglobulin gamma. This

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cleavage is dependent on protein kinase C and tyrosine kinases and can be blocked by protease inhibitors. The shedding is inhibited by the tissue inhibitor of metalloproteinase TIMP3, and thus probably induced by membrane-bound metalloproteinases ADAMs. Intravenous LPS produces a rapid rise of sCD163 in plasma of patient as it induces metalloproteinase- mediated shedding from monocytes surface.

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Plate:	Pre-coated
Protocol:	This particular immunoassay utilizes the quantitative technique of a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a sandwich format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on the Human CD163 cytokine while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and sandwiching of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'- Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.
Sample Preparation:	If samples are to be used within 24 hours, aliquot and store at 4 °C. If samples are to be used over a long period of time, aliquot and store between -20 °C and -80 °C, depending on the duration of storage. Note: Samples containing a visible precipitate or pellet must be clarified prior to use in the assay. Caution: Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples. • Cell Lysate and Supernatants: Remove large cell components via centrifugation and perform the assay. Cell lysates and

supernatants require a dilution using Assay Diluent. A serial dilution may be performed to determine a suitable dilution factor for the sample. For future use of the sample, follow the sample storage guidelines stated above.

- Serum:
  Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Serum samples require at least a 1:50 dilution using Assay Diluent. For future use of the sample, follow the storage guidelines above.
- Plasma:
  Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Plasma samples require at least a 1:50 dilution using Assay Diluent. Afterwards, perform the assay or for future use of the sample, follow the storage guidelines stated above.

Assay Procedure:

Note: If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.

Note: Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom.

Reconstitution of Provided Materials:

1. Reconstitute the Biotin-Conjugated Detection Antibody in 67 µL of ddH□O for a concentration of 180 µg/ml.
2. Reconstitute the Protein Standard in 100 µL of ddH□O for a concentration of 340 ng/ml.
3. Dilute the 50 mL of 10x Wash Buffer in 450 mL of ddH2O for 500 mL of 1x Wash Buffer.

Addition of Known Standard and Unknown Sample to Immunoassay:

The OmniKine™ Human CD163 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human CD163 proteins

Calculation of Results:

Generation of Standard Curve and Interpretation of Data

1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.

2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4- Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or trend-line through the plotted points via regression analysis. Note: Shown on the next page is an example of typical

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	data produced by analysis of the standard sample.
Restrictions:	For Research Use only

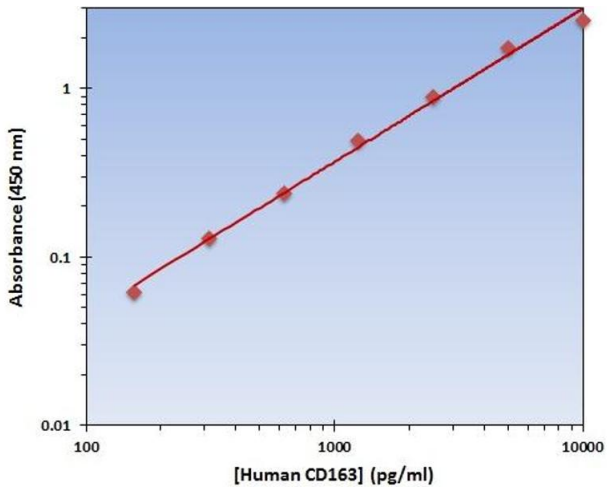
Handling

Precaution of Use:	Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment. Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
Handling Advice:	This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind. Materials included in this kit should NOT be used past the expiration date on the kit label. Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits. Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided. The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment. Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
Storage:	4 °C
Storage Comment:	Note: If used frequently, reagents may be stored at 4 °C. • Unopened Kits: Store at 4 °C for 6 months. • Microstrips Coated w/ Capture Antibody, 400x Streptavidin-HRP Wash Buffer (10x), Assay Diluent Ready-to-Use Substrate, Stop Solution: 6 Months at 4 °C • Protein Standard, Biotinylated Detection Antibody: Lyophilized: 6 Months (if Reconstituted: 1

Handling

Month) at 4 °C

Images



ELISA

Image 1. This is an example of what a typical standard curve will look like. You must make your own standard curve. Do not use this example as your own standard curve.