Datasheet for ABIN1440231

Complement C8 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Complement C8 (C8)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	~ 0.6 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Complement C8 ELISA kit is designed for detection of C8 in human plasma, serum, saliva, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C8 in less than 4 hours. A polyclonal antibody specific for C8 has been pre-coated onto a microplate. C8 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C8, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax
Sample Type:	Serum, Milk, Saliva, Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	Cross-Reactivity: Monkey <20%, Rat 1%

Product Details

Characteristics:	Standard Added Value: 1.5 - 15 ng/mL
Components:	C8 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against C8. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. C8 Standard: Human C8 in a buffered protein base (40 ng, lyophilized). Biotinylated C8 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against C8 (140 µL). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 mL). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 mL, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µL). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 mL). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 mL).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel pipettes). Deionized or distilled reagent grade water.

Target Details

Target:	Complement C8 (C8)
Alternative Name:	Complement C8 (C8 Products)
Background:	Complement Component 8 (C8) is a 150-kDa complex composed of three genetically distinct subunits: C8alpha (64 kDa), C8beta (64 kDa), and C8gamma (22 kDa). C8alpha and C8beta are highly homologous to each other and to C6, C7 and C9, and contain a common membrane attack complex/perforin (MACPF) domain. C8gamma has a lipocalin fold and shares no homology with any other complement protein. C8 plays a central role in membrane attack complex MAC assembly by coordinating the interaction with complement proteins C5b-7 and the pore-forming protein C9 on pathogen membranes. It is also the first component to penetrate the lipid bilayer. C8 deficiency exhibits an increased susceptibility to Neisseria meningitidis infections and recurrent meningococcal disease.

Application Details

Sample Volume:	50 µL

Application Details

Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	Add 50 µL of standard/samples per well. Incubate 2 hours. Wash, then add 50 µL of biotinylated antibody per well. Incubate 1 hour. Wash, then add 50 µL of SP per well. Incubate 30 minutes. Wash, then add 50 µL of TMB per well. Incubate 15 minutes. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. 4 Standard Curve: Reconstitute the 40 ng of human C8 standard with 1 mL of EIA Diluent to generate a stock standard solution of 40 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the C8 standard solution 1:2 with equal volume of EIA Diluent to produce 20, 10, 5, 2.5, 1.25, and 0.625 ng/mL. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within the next 30 days. Biotinylated C8 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the wash buffer 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Preparation:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:10000 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes. Remove serum and dilute samples 1:10000 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and

	assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Milk: Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:20 into EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of C8 Biotin to each well and incubate for 1 hour. Wash the microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for approximately 15 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four- parameter logistic curve-fit. Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.7% and 7.3% respectively.

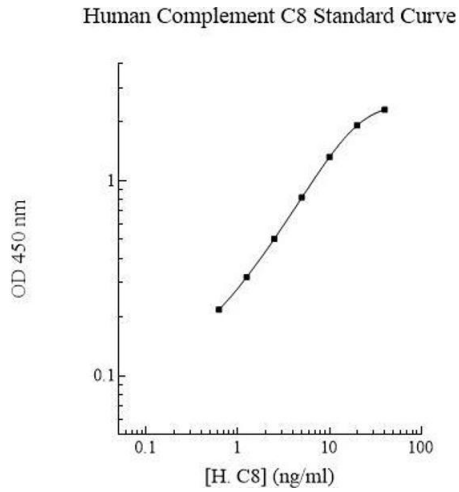
Application Details

Restrictions:	For Research Use only

Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The kit should not be used beyond the expiration date. The Stop Solution is an acid solution.
Storage:	4 °C/-20 °C
Storage Comment:	Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and biotinylated antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 1 month in a vacuum desiccator. 3 Diluent (1x) may be stored for up to 1 month at 2-8°C. Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Images



ELISA

Image 1.