Datasheet for ABIN1440229

C6 ELISA Kit

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Overview

Quantity:	96 tests
Target:	C6
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.7 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Complement C6 ELISA kit is designed for detection of C6 in human plasma, serum, saliva, urine, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures C6 in less than 4 hours. A polyclonal antibody specific for C6 has been pre-coated onto a microplate. C6 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for C6, 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, Urine, Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	Cross-Reactivity: Monkey <20%

Product Details

Characteristics:	Standard Added Value: 2.5 - 25 ng
Components:	Human C6 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human C6. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human C6 Standard: Human C6 in a buffered protein base (200 ng, lyophilized). Biotinylated Human C6 Antibody (100x): A 100-fold biotinylated polyclonal antibody against human C6 (80 µL). MIX 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). Deionized or distilled reagent grade water.

Target Details

Target:	C6
Alternative Name:	Complement C6 (C6 Products)
Background:	Human Complement Component 6 (C6) is a single-chain glycoprotein consisting of 913 amino acid residues with a molecular mass of about 102 kDa. C6 is a part of the lytic membrane attack complex during complement activation. Cleavage of C5 into C5a and C5b by C5 convertase triggers binding of plasma C6 to C5b. Once the C5b-6 complex forms, C7, C8, and C9 add sequentially to create a transmembrane channel structure. Complete deficiency of C6 (C6Q0) leads to an increased susceptibility to Neisseria meningitidis infections and recurrent meningococcal disease. In animal models, genetic C6 deficiency accelerates axonal regeneration and reduces atherosclerosis.

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h

Application Details

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 Chromogen Substrate 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. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 200 ng of Human C6 Standard with 4 mL of MIX Diluent to generate a solution of 50 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 standard solution (50 ng/mL) 1:2 with MIX Diluent to produce 25, 12.5, 6.25, 3.13, 1.56 and 0.78 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C. Biotinylated Human C6 Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX 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 Concentrate 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 MIX 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 MIX Diluent and assay. Store samples 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. Dilute samples 1:10000 into MIX Diluent and assay. Store samples 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. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles. Urine: Collect urine 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.

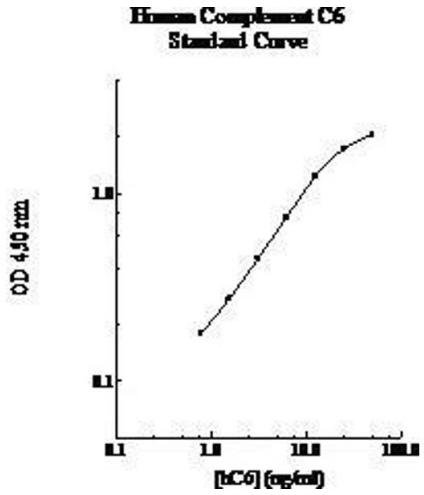
	Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:40 into MIX Diluent and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze- thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:2 into MIX Diluent and assay. 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 Human C6 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 material 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 material to completely remove the liquid. Add 50 µL of Biotinylated Human C6 Antibody 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 about 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 four-parameter or log-log logistic curve-fit.

Application Details

	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 4.1% and 7.1% respectively.
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 acidic 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. 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.



ELISA

Image 1.