Datasheet for ABIN612679

Complement C4 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Complement C4 (C4)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.08 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Complement C4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human complement C4 in urine, saliva and cell culture supernatants
Brand:	AssayMax
Sample Type:	Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Components:	Human Complement C4 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C4. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Complement C4 Standard: Human Complement C4 in a buffered protein base (1.6 µg, lyophilized). Biotinylated Complement C4 Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Complement C4 (80µl). 1 MIx Diluent

Product Details

	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 (80µl). 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, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	Complement C4 (C4)
Alternative Name:	Complement C4 (C4 Products)
Background:	Complement protein C4 is the second component to react in the complement sequence. It is a beta-globulin with a sedimentation coefficient of 18.7 and a molecular weight of 240,000. The C4 component participates in the initial step of activation of classical complement pathway. Lower levels of complement C4 in serum was associated with primary biliary cirrhosis , human systemic lupus erythematosus , and chronic liver disease.
Pathways:	Complement System

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures human complement C4 in less than 4 hours. A polyclonal antibody specific for human complement C4 has been pre-coated onto a 96-well microplate with removable strips. Complement C4 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Complement C4, 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.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. MIx

	Diluent Concentrate (10x): Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 1.6 g of Complement C4 Standard with 5 ml of MIx Diluent to generate a stock of 320 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The stock standard (320 ng/ml) should be further diluted 1:16 with MIx to generate a standard solution of 20 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (20 ng/ml) 1:4 with MIx Diluent to produce 5, 1.25, 0.313 and 0.078 ng/ml solutions. MIx Diluent serves as the zero standard (0 g/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Complement C4] (ng/ml) P1 Stock (320 ng/ml) + 15 part MIx Diluent 20.000 P2 1 part P1 + 3 parts MIx Diluent 5.000 P3 1 part P2 + 3 parts MIx Diluent 1.250 P4 1 part P3 + 3 parts MIx Diluent 0.313 P5 1 part P4 + 3 parts MIx Diluent 0.078 P6 MIx Diluent 0.000 Biotinylated Complement C4 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): 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 Collection:	Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:8 into MIx Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles Saliva: Collect saliva using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:200 into MIx Diluent. 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 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. 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 desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of Complement C4 standard or sample per well. Cover wells with a sealing tape and incubate for two 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 it 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, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated Complement C4 Antibody to each well and incubate for one hour. Wash a microplate as described above. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well

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	and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap 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. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.6 % and 7.3 % respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened MIx Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

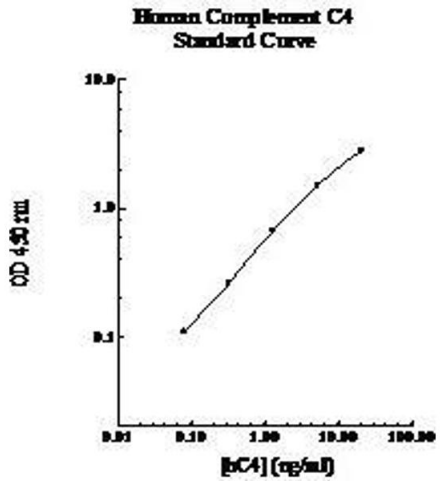
Publications

Product cited in:	Ajona, Razquin, Pastor, Pajares, Garcia, Cardenal, Fleischhacker, Lozano, Zulueta, Schmidt, Nadal, Paz-Ares, Montuenga, Pio: "Elevated levels of the complement activation product C4d in bronchial fluids for the diagnosis of lung cancer." in: **PLoS ONE**, Vol. 10, Issue 3, pp. e0119878, (2015) (PubMed).

Publications

Koehler, Swain, Sanderson, Krishnan, Watt, Charlton: "Growth hormone, dehydroepiandrosterone and adiponectin levels in non-alcoholic steatohepatitis: an endocrine signature for advanced fibrosis in obese patients." in: **Liver international : official journal of the International Association for the Study of the Liver**, Vol. 32, Issue 2, pp. 279-86, (2012) (PubMed).

Images



ELISA

Image 1.