Datasheet for ABIN1440235

Complement Factor B ELISA Kit

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Overview

Quantity:	96 tests
Target:	Complement Factor B (CFB)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	~2.5 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Factor B (FB) ELISA kit is designed for detection of human factor B in plasma, serum, saliva, urine, milk and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures Factor B in less than 4 hours. An antibody specific for Factor B has been pre-coated onto a 96-well microplate with removable strips. Factor B in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Factor B, 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

Product Details

Cross-Reactivity (Details):	Cross-Reactivity: Monkey 1%, Mouse 1% 10% FBS in culture media will not affect the assay.
Characteristics:	Standard Added Value: 10 - 100 ng/mL
Components:	Factor B Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Factor B. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. AssayMax Human Factor B ELISA Kit Catalog No. EF7001-1 This protocol serves as an example for the above. Do not use this protocol in conjunction with any purchased kit. 2 Factor B Standard: Human FB in a buffered protein base (1280 ng, lyophilized). Biotinylated FB Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against FB (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) Deionized or distilled reagent grade water

Target Details

Target:	Complement Factor B (CFB)
Alternative Name:	Complement Factor B (CFB Products)
Background:	Complement Factor B (FB) is a component of the alternative pathway of complement activation. The zymogen circulates in the blood as a 93 kDa single chain glycoprotein with 739 amino acids. In the presence of C3b, it is cleaved by factor D into a 30 kDa N terminal noncatalytic Ba fragment and a 63 kDa C terminal catalytic Bb fragment. The active subunit Bb associates with C3b to form the alternative pathway C3 convertase. FB plays a major role in the initiation of the alternative pathway and in amplification of C3 cleavage. The polymorphism of FB influences C3 convertase formation, and is associated with age-related macular degeneration and polypoidal choroidal vasculopathy.

Target Details

Pathways:	Complement System, Proton Transport, Ribonucleoside Biosynthetic Process

Application Details

Assay Time:	< 4 h
Plate:	Pre-coated
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 the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 1280 ng of human FB Standard with 4 mL of EIA Diluent to generate a stock solution of 320 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 stock solution (320 ng/mL) 1:2 with equal volume of EIA Diluent to produce 160, 80, 40, 20, 10, 5 and 2.5 ng/mL. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days. Biotinylated FB 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 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 EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Preparation:	Plasma: Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:5000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA can also be used as anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum, dilute samples 1:5000 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Collect cell culture media and centrifuge at 2000 x g for 10 minutes at 40C to remove debris. 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

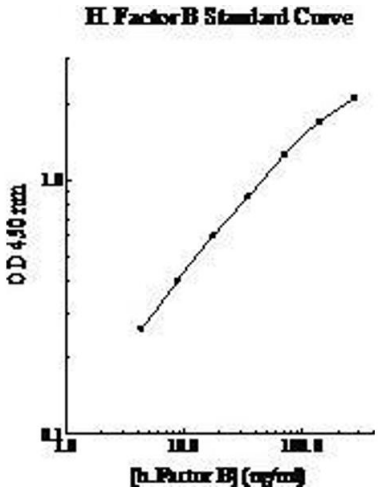
	assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 3 Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute milk samples 1:20 into EIA Diluent. The undiluted samples can be stored 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. 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 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 FB Antibody to each well and incubate for one 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 develop. 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

Application Details

	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 5.0 % and 7.2 % 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 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 Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. 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.