Datasheet for ABIN612692

F12 ELISA Kit

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Overview

Quantity:	96 tests
Target:	F12
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	50 pg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Factor xII (FxII) ELISA kit is designed for detection of human factor xII in plasma, serum, milk, urine, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	FxII Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against FxII. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. FxII Standard: Human FxII in a buffered protein base (100 ng, lyophilized). Biotinylated FxII Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against FxII (80 l). 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). Streptavidin-Peroxidase Conjugate (SP Conjugate): A

Product Details

	100-fold concentrated (80 l). 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).

Target Details

Target:	F12
Alternative Name:	Factor xII (FxII) (F12 Products)
Background:	Human coagulation factor xII (FxII), Hageman factor, is a plasma serine protease existing in the zymogen form. Upon contacting with negatively charged artificial or biologic surfaces, FxII is autoactivated into FxIIa that initiates intrinsic blood coagulation, fibrinolysis, and activation of the inflammatory kallikrein-kinin and complement systems (1 - 3). FxII has 615 amino acids, weighs 80 kDa and circulates in normal plasma at a concentration of 30 µg/ml (4-5). It is a multidomain protein with structure similarity to EGF, single chain urokinase, and tissue plasminogen activator. In the intravascular compartment, FxII binds to endothelial cell urokinase plasminogen activator receptor, cytokeratin 1, and the complement receptor. FxII deficiency or blockade protects from cerebral ischemia without overtly affecting hemostasis. FxII inhibition could be a novel target for safer anticoagulation and stroke prevention without the side effect of increased bleeding (7-8).
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 FxII in less than 4 hours. A murine antibody specific for FxII has been pre-coated onto a 96-well microplate with removable strips. FxII in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for FxII, 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. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. FxII Standard: Reconstitute the 100 ng of human FxII Standard with 1 ml of EIA Diluent to generate a Standard solution of 100 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (100 ng/ml) twofold with equal volume of EIA Diluent to produce 50, 25, 12.5, 6.25 and 3.13 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [FxII] (ng/ml) P1 1 part Standard (100 ng/ml) 100.0 P2 1 part P1 + 1 part EIA Diluent 50.00 P3 1 part P2 + 1 part EIA Diluent 25.00 P4 1 part P3 + 1 part EIA Diluent 12.50 P5 1 part P4 + 1 part EIA Diluent 6.25 P6 1 part P5 + 1 part EIA Diluent 3.13 P7 EIA Diluent 0.00 Biotinylated FxII Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA 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 EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and collect supernatants. Dilute samples 1:1000 into EIA Diluent and assay as follows: add 10 l of sample to 990 l of EIA Diluent (1:100) to make Solution A, then add 50 l of Solution A to 450 l of EIA Diluent (1:10) to make a final working solution (1:1000). 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 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 and assay. Dilute samples 1:1000 into EIA Diluent and assay as follows: add 10 l of sample to 990 l of EIA Diluent (1:100) to make Solution A, then add 50 l of Solution A to 450 l of EIA Diluent (1:10) to make a final working solution (1:1000). 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. Collect supernatants 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 tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 2 Milk: Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes. Milk dilution is suggested at 1:4 in EIA Diluent, however, the user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months.

Application Details

	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 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. Add 50 µL of Biotinylated FxII Antibody to each well and incubate for 1 hour. Wash a 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 a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal 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. 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 5.6% and 6.8% 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

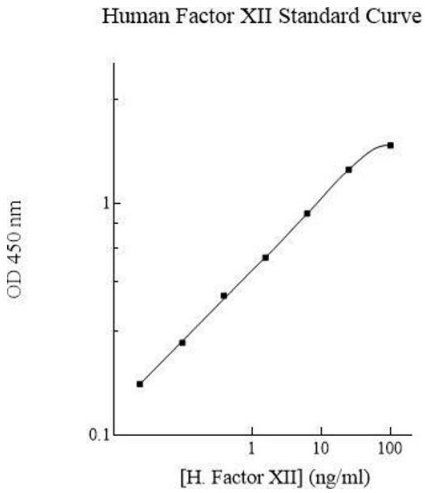
Handling

	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.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened EIA 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. Other Supplies required Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 l, 20-200 l, and multiple channel pipettes). Deionized or distilled reagent grade water.

Publications

Product cited in:	Gao, Lu, Xiao, Yang, Chen, Zhou, Wen, Li, Wu, Jiang, Liu, Zhao: "β-Eliminative depolymerization of the fucosylated chondroitin sulfate and anticoagulant activities of resulting fragments." in: **Carbohydrate polymers**, Vol. 127, pp. 427-37, (2015) (PubMed). Bhargava, Becker, Viken, Jagtap, Dey, Steinbach, Wu, Kumar, Bitterman, Ingbar, Wendt: " Proteomic profiles in acute respiratory distress syndrome differentiates survivors from non-survivors." in: **PLoS ONE**, Vol. 9, Issue 10, pp. e109713, (2014) (PubMed).

Images



ELISA

Image 1.