Datasheet for ABIN612691

Factor XI ELISA Kit

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Overview

Quantity:	96 tests
Target:	Factor XI (F11)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.7 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Factor xI (FxI) ELISA kit is designed for detection of human factor xI in plasma, serum, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	FxI Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against FxI. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. FxI Standard: Human FxI in a buffered protein base (6.4 µg, lyophilized). Biotinylated FxI Antibody (80x): A 80-fold concentrated biotinylated polyclonal antibody against FxI (100 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, 2 bottles). Streptavidin-Peroxidase Conjugate (SP

Product Details

	Conjugate): A 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:	Factor XI (F11)
Alternative Name:	Factor xI (FxI) (F11 Products)
Background:	Human coagulation factor xI (FxI), also called plasma thromboplastin antecedent, is a serine protease important for initiating the contact activation or intrinsic pathway of blood coagulation. FxI is present in plasma as a homodimer zymogen consisting of two identical polypeptide chains of 607 amino acids and 80 kDa each. FxI circulates in normal plasma at a concentration of 5 g/ml. It is activated to form FxIa not only by factor xIIa through the contact pathway, but also by thrombin through feedback activation linking to tissue factor or extrinsic pathway. FxIa in turn cleaves factor Ix and triggers a cascade events converting fibrinogen to a stable cross-linked fibrin clot formation (1-3). FxI also plays a role in the prevention of clot lysis from fibrinolysis. Congenital FxI deficiency is accompanied by mild and injury-related bleeding. Severe FxI deficiency is linked to low occurrence of ischemic stroke or venous thrombosis. In contrast, elevated FxI activity is a risk factor for stroke, venous thrombosis and coronary artery disease (6- 8). FxI is a new target for the treatment and prevention of thromboembolism.

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 FxI in less than 4 hours. A polyclonal antibody specific for FxI has been pre-coated onto a 96-well microplate with removable strips. FxI in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for FxI, 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. FxI Standard: Reconstitute the 6.4 g of human FxI Standard with 4 ml of EIA Diluent to generate a stock solution of 1.6 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Dilute a stock solution (1.6 g/ml) 1:8 to prepare a standard solution of 200 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the Standard solution (200 ng/ml) 1:4 with EIA Diluent to produce standard solution of 50, 12.5, 3.13 and 0.78 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [FxI] (ng/ml) P1 1 part Standard (1.6 g/ml) + 7 parts EIA Diluent 200.00 P2 1 part P1 + 3 parts EIA Diluent 50.00 P3 1 part P2 + 3 parts EIA Diluent 12.50 P4 1 part P3 + 3 parts EIA Diluent 3.13 P5 1 part P4 + 3 parts EIA Diluent 0.78 P6 EIA Diluent 0.00 Biotinylated FxI Antibody (80x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 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 1:600 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 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 1:600 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. 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. 2
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. If using a machine wash

Application Details

	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 FxI 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 four-parameter or log-log 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 4.9% and 7.1% respectively.
Restrictions:	For Research Use only

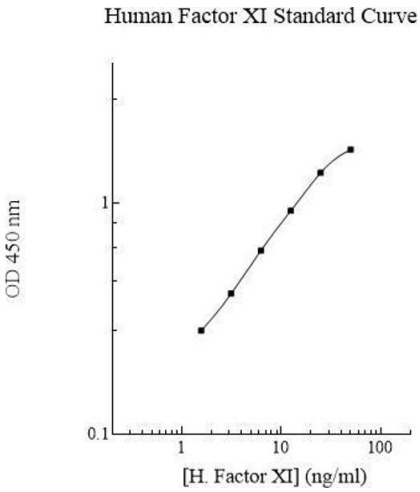
Handling

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. 1 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.

Handling

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.

Images



ELISA

Image 1.