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Datasheet for ABIN5564543 Factor XI ELISA Kit

Overview

Quantity:	96 tests
Target:	Factor XI (F11)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1.562-50 ng/mL
Minimum Detection Limit:	1.562 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Factor XI (FXI) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human factor XI in plasma, serum, and cell culture samples. 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.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human Factor XI Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against FXI. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Factor XI Standard: Human FXI in a buffered protein base (50 ng, lyophilized, 2 vials). Biotinylated Human Factor XI Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against FXI (140 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 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). Positive Control: 1 vial, See Protocol CEF10111 Negative Control: 1 vial, See Protocol CEF10112
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C)

Target Details

Target:	Factor XI (F11)
Alternative Name:	Factor XI (Factor 11) (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 µg/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 event 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 (4). 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 (5). 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.
Gene ID:	2160
UniProt:	P03951

Application Details

Plate:	Pre-coated
Protocol:	Add 50 µL of Standard/ Sample 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 Conjugate 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. Assay Template 1 2 1 1 1 0 9 8 7 6 5 4 3 2 1 A B C D E F G H 1 AssayMax Human Factor XI ELISA Kit Catalog No. EF1011-7 Sample protocol for reference only
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 Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8 °C. Human Factor XI Standard: Reconstitute the 50 ng (6.2 mU) of Human Factor XI Standard with 1 mL of EIA Diluent to generate a 50 ng/mL (6.2 mU/mL) standard solution. 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 EIA Diluent to produce standard solution of 25, 12.5, 6.25, 3.125, and 1.562 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20 °C and used within 5 days. 4 Standard Point Dilution [FXI] (ng/mL) [FXI] (mU/mL) P1 Standard (50 ng/mL) 50.00 6.200 P2 1 part P1 + 1 part EIA Diluent 25.00 3.100 P3 1 part P2 + 1 part EIA Diluent 12.50 1.550 P4 1 part P3 + 1 part EIA Diluent 6.250 0.775 P5 1 part P4 + 1 part EIA Diluent 3.125 0.388 P6 1 part P5 + 1 part EIA Diluent 1.562 0.194 P7 EIA Diluent 0.000 0.000 Biotinylated Human Factor XI 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.
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °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 l of Human Factor XI Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l 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

Application Details

machine, wash six times with 300 l 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 l of Biotinylated Human Factor XI Antibody to each well and incubate for 1 hour. Wash the microplate as described above. 5 Add 50 l 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 l of Chromogen Substrate per well and incubate for 15 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 l 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:	<ul style="list-style-type: none">• 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 (OD) 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.
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Restrictions:	For Research Use only
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Handling

Handling Advice:	Prepare all reagents (working diluent buffer, wash buffer, standards, positive control, negative control, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. 2 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. This kit is for research use only. The kit should not be used beyond the expiration date. The Stop Solution is an acidic solution.
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Storage:	4 °C,-20 °C
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Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures 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.
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Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. 3 Store Standard, Positive Control, and Negative Control at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent. Other Supplies required Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 l, 20-200 l, 200-1000 l, and multiple channel). Deionized or distilled reagent grade water. Sample Collection, Preparation and Storage 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 and collect supernatants. Dilute samples 1:600 into EIA Diluent or within the range of 1:300 to 1:1200, and assay. 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 an anticoagulant). Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 1:600 into EIA Diluent or within the range of 1:300 to 1:1200, 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 3000 x g for 10 minutes at 4°C to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.