Datasheet for ABIN612690

Coagulation Factor X ELISA Kit

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Overview

Quantity:	96 tests
Target:	Coagulation Factor X (F10)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	1 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Factor x (Fx) ELISA kit is designed for detection of human factor x in plasma, serum, milk, urine, saliva, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Fx Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human Fx. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Fx Standard: Plasma human Fx in a buffered protein base (400 ng, lyophilized). 1 Biotinylated Fx Antibody (80x): A 80-fold biotinylated polyclonal antibody against H. Fx (100 l). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold

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	concentrated buffered surfactant (30 ml, 2 bottles). 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:	Coagulation Factor X (F10)
Abstract:	F10 Products
Background:	Factor x (Fx) is a plasma serine protease zymogen involved in the blood coagulation cascade. Fx is purified from plasma as a two-chain protein consisting of a 45 kDa heavy chain and a 17 kDa light chain. The Fx heavy chain is cleaved during coagulation by several different proteases including the intrinsic xase complex, the Fx-activating enzyme from Russell's viper venom (RVV) and trypsin, and also by extrinsic (tissue factor/factor VIIa) pathway to give an active enzyme Fxa. Fxa as the activator of prothrombin occupies a central position linking the two blood coagulation pathways (1 - 4).

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Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures Fx in less than 4 hours. A monoclonal antibody specific for Fx has been pre-coated onto a 96-well microplate with removable strips. Fx in standards and samples is sandwiched by the immobilized antibody and the peroxidase conjugated polyclonal antibody specific for Fx. 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 400 ng of human Fx Standard with 2 ml of MIx Diluent to generate a Stock solution of 200 ng/ml. Allow the standard to sit for 10 minutes

	with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by diluting the Stock solution (200 ng/ml) 1:2 with MIx Diluent to generate solution of 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 ng/ml. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Fx] (ng/ml) P1 1 part Stock (200 ng/ml) + 1 part MIx Diluent 100.000 P2 1 part P1 + 1 part MIx Diluent 50.000 P3 1 part P2 + 1 part MIx Diluent 25.000 P4 1 part P3 + 1 part MIx Diluent 12.500 P5 1 part P4 + 1 part MIx Diluent 6.250 P6 1 part P5 + 1 part MIx Diluent 3.125 P7 1 part P6 + 1 part MIx Diluent 1.563 P8 MIx Diluent 0.000 Biotinylated Fx Antibody (80x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 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:	Plasma: Collect plasma using 3.8% sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes. Dilute plasma 1:800 into MIx 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 1:800 into MIx Diluent. 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. Store samples at
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 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 Fx 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 approximately 20 minutes or till the optimal

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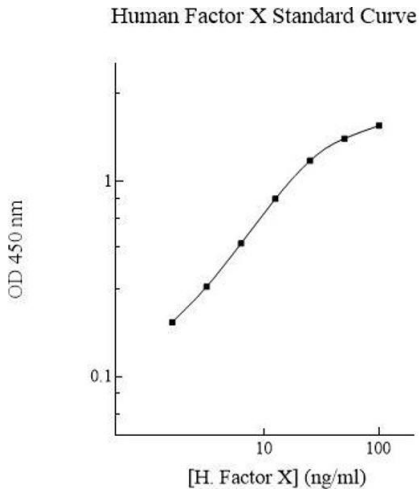
	blue color density develop. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength 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 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 4.8 % 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. 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.



ELISA

Image 1.