Datasheet for ABIN612697

Factor VII ELISA Kit

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Overview

Quantity:	96 tests
Target:	Factor VII (F7)
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	1.4 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Factor VII (FVII) ELISA kit is designed for detection of human factor VII and factor VIIa in plasma, serum, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The minimum detectable dose of human FVII is typically ~1.4 ng/ml.
Components:	FVII Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FVII. 1 Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. FVII Standard: Human FVII in a buffered protein base (270 ng, lyophilized). Biotinylated FVII Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against FVII (140µl). MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A

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	20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). 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:	Factor VII (F7)
Alternative Name:	Factor VII (FVII) (F7 Products)
Background:	Factor VII (FVII) is a vitamin K-dependent plasma glycoprotein that is synthesized in the liver and circulates in blood as a single-chain inactive zymogen with a molecular mass of 50 kDa. Upon tissue damage and vascular injury, the cell surface receptor and cofactor tissue factor binds and allosterically activates FVII to its active form, FVIIa. The tissue factor/FVIIa complex catalyzes the conversion of both factor Ix to factor Ixa and factor x to factor xa to initiate coagulation via the extrinsic pathway. Very low levels of FVII are associated with severe coagulation disorders. Elevated plasma levels of FVII coagulant activity constitute an independent risk factor for fatal outcomes of coronary heart disease in middle-aged men.
Pathways:	Response to Growth Hormone Stimulus, Platelet-derived growth Factor Receptor Signaling

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 total FVII in less than 4 hours. A polyclonal antibody specific for FVII has been pre-coated onto a 96-well microplate with removable strips. FVII in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for FVII, 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. MIx Diluent Concentrate (10x): Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. 2 Standard Curve: Reconstitute the 270 ng of human FVII Standard with 3 ml of MIx Diluent to generate a 90 ng/ml stock 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 stock solution (90 ng/ml) 1:2 with equal volume of MIx Diluent to produce 45, 22.5, 11.25, 5.625, 2.813, and 1.406 ng/ml. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [FVII] (ng/ml) P1 1 part Standard (90 ng/ml) 90.000 P2 1 part P1 + 1 part MIx Diluent 45.000 P3 1 part P2 + 1 part MIx Diluent 22.500 P4 1 part P3 + 1 part MIx Diluent 11.250 P5 1 part P4 + 1 part MIx Diluent 5.625 P6 1 part P5 + 1 part MIx Diluent 2.813 P7 1 part P6 + 1 part MIx Diluent 1.406 P8 MIx Diluent 0.000 Biotinylated FVII Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 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 and assay. Dilute samples 1:20 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 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 1:20 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. The samples can be stored at -20°C or below. 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

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	absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated FVII 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 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 5.0 % and 7.2 % respectively.
Restrictions:	For Research Use only

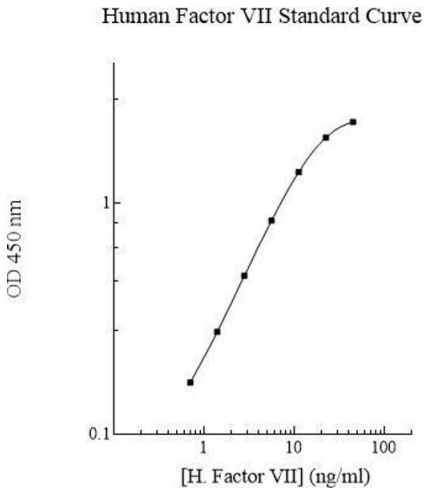
Handling

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.
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

Handling

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

Images



ELISA

Image 1.