

Datasheet for ABIN612689

Coagulation Factor IX ELISA Kit[Go to Product page](#)**1** Image

Overview

Quantity: 96 tests

Target: Coagulation Factor IX (F9)

Reactivity: Human

Method Type: Sandwich ELISA

Minimum Detection Limit: 1.6 ng/mL

Application: ELISA

Product Details

Purpose: The AssayMax Human Factor Ix (Flx) ELISA kit is designed for detection of human factor Ix in plasma and cell culture supernatants

Brand: AssayMax

Sample Type: Plasma, Cell Culture Supernatant

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: 10% FBS in culture media will not affect the assay.

Components: Flx Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Flx. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Flx Standard: Human Flx in a buffered protein base (2 µg, lyophilized). Biotinylated Flx Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Flx (80 l). EIA 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). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). 1 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: Coagulation Factor IX (F9)

Alternative Name: Factor IX (Flx) ([F9 Products](#))

Background: Factor Ix (Flx) is zymogens of plasma serine proteases required for normal hemostasis. Flx and Fx were activated by tissue factor (TF) and factor VIIa (FVIIa) complexes and initiates coagulation resulting in thrombin formation. Hemophilia B is an x-linked bleeding disorder that results from a deficiency in functional coagulation factor Ix (hFlx). On the other hand, increased plasma level of Flx was reported to be independent risk factor of venous thromboembolism (VTE).

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 Flx in less than 4 hours. A polyclonal antibody specific for Flx has been pre-coated onto a 96-well microplate with removable strips. Flx, in standards and samples, is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Flx, 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. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Flx Standard: Reconstitute the 2 g of human Flx Standard with 5.0 ml of EIA Diluent to generate a stock standard solution of 400 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The stock standard solution can be further dilute 1:4 with EIA to generate standard solution of 100 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the Standard solution (100 ng/ml) 1:2 with EIA Diluent to produce 50,

25, 12.5, 6.25, 3.13 and 1.56 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Flx] (ng/ml) P1 1 part Standard (400 ng/ml) + 3 part EIA Diluent 100.00 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 1 part P6 + 1 part EIA Diluent 1.56 P8 EIA Diluent 0.00 Biotinylated Flx 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 1:400 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. (EDTA or Heparin can also be used as anticoagulant.) Cell Culture Supernatants: Collect cell culture media and centrifuge at 2000 x g for 10 minutes at 4°C 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.

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 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 Flx Antibody to each well and incubate for one 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 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 µL of Stop Solution to each well. The color will

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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 5.7 % and 7.2 % respectively.

Restrictions: For Research Use only

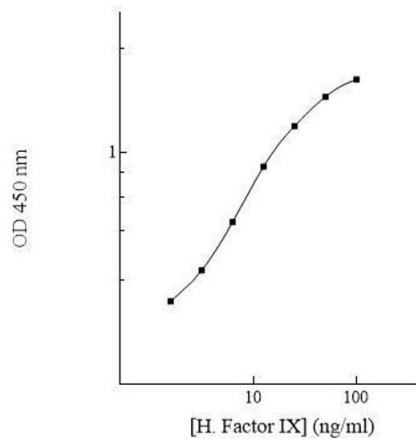
Handling

Handling Advice: 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, 200-1000 l and multiple channel pipettes). Deionized or distilled reagent grade water.

Human Factor IX Standard Curve



ELISA

Image 1.