Version 15-12/24

User's Manual

F10 (Human) ELISA Kit

REF **DEIA3407**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

F10 (Human) ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of human F10.

General Description

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 the extrinsic (tissue factor/factor VIIa) pathway to give an active FXa enzyme. FXa, as the activator of prothrombin, occupies a central position linking the two blood coagulation pathways (1-4).

Principles of Testing

The F10 (Human) ELISA Kit is designed for detection of FX in human plasma, serum, milk, urine, saliva, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human FX in approximately 4 hours. A monoclonal antibody specific for human 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 a biotinylated polyclonal antibody specific for human FX, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Human Factor X Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human FX. 96 (8×12) wells
2. Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. 3 slices
3. Human Factor X Standard: Human FX in a buffered protein base (lyophilized). 170 ng
4. Biotinylated Human Factor X antibody (50×): A 50-fold concentrates biotinylated polyclonal antibody against human FX. 120 µL
5. MIX Diluent Concentrate (10×): A 10-fold concentrated buffered protein base. 30 mL
6. Wash Buffer Concentrate (20×): A 20-fold concentrated buffered surfactant. 30 mL × 2
7. SP Conjugate (100×): A 100-fold concentrate. 80 µL
8. Chromogen Substrate (1×): A stabilized peroxidase chromogen substrate tetramethylbenzidine. 8 mL
9. Stop Solution (1×): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction. 12 mL

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel)
3. Deionized or distilled reagent grade water

Storage

1. Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
2. Store SP Conjugate and Biotinylated Antibody at -20°C.
3. Store Microplate, Diluent Concentrate (10×), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
4. 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.
5. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Specimen Collection And Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 × g for 10 minutes and collect plasma. An 800-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. 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 × g for 10 minutes and remove serum. An 800-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 × g for 10 minutes. A 2-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample pot. Centrifuge samples at 800 × g for 10 minutes. The sample is suggested for use at 1×; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 × g for 10 minutes. The sample is suggested for use at 1×; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 × g for 10 minutes. The sample is suggested for use at 1×; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Note: Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A. 4 µL sample: 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A: 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A. 4 µL sample: 396 µL buffer (100x) B. 24 µL of A: 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A: 396 µL buffer (100x) C. 24 µL of B: 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Reagent Preparation

Freshly dilute all reagents and bring all reagents to room temperature before use.

1. MIX Diluent Concentrate (10×): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1× solution. Store for up to 30 days at 2-8°C.
2. Human Factor X Standard: Reconstitute the Human Factor X Standard (170 ng, 20.4 mIU) with 1.7 mL of MIX Diluent to generate a 100 ng/mL (12 mIU/mL) standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (100 ng/mL) 2-fold with equal volume of MIX Diluent to produce 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[FX] (ng/mL)	[FX] (mIU/mL)
P1	1 part Standard + 1 part MIX Diluent	50	6.00
P2	1 part P1 + 1 part MIX Diluent	25	3.00
P3	1 part P2 + 1 part MIX Diluent	12.5	1.50
P4	1 part P3 + 1 part MIX Diluent	6.25	0.75
P5	1 part P4 + 1 part MIX Diluent	3.125	0.375
P6	1 part P5 + 1 part MIX Diluent	1.563	0.188
P7	1 part P6 + 1 part MIX Diluent	0.781	0.094
P8	MIX Diluent	0.0	0.0

3. Biotinylated Human Factor X Antibody (50×): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1× solution. The undiluted antibody should be stored at -20°C.
4. Wash Buffer Concentrate (20×): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1× solution.

5. SP Conjugate (100×): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1× solution. The undiluted conjugate should be stored at -20°C.

Assay Procedure

1. 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).
2. 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.
3. Add 50 µL of Human Factor X Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human Factor X Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. 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

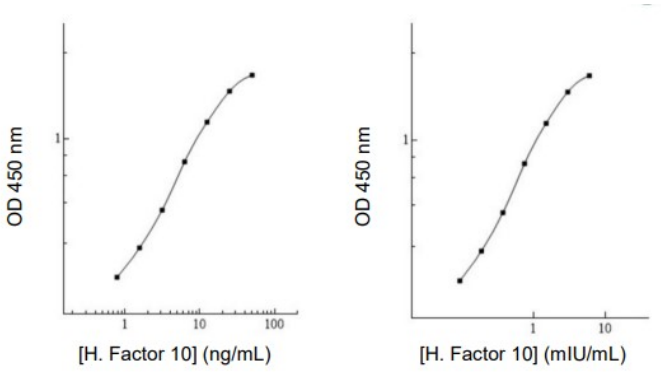
1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Standard Curve

The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	50.00	2.219 2.173	2.151
P2	25.00	1.741 1.803	1.772
P3	12.50	1.239 1.201	1.220
P4	6.250	0.771 0.737	0.754
P5	3.125	0.408 0.434	0.421
P6	1.563	0.273 0.261	0.267
P7	0.781	0.184 0.190	0.187
P8	0.000	0.109 0.111	0.110
Sample: Pooled Normal Sodium Citrate Plasma (800x)		1.253 1.271	1.262

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Precision

Intra-assay precision was determined by testing three plasma samples twenty times in one assay.

Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.7%	4.5%	5.3%	8.9%	8.6%	9.3%
Average CV (%)	4.8%			8.9%		

Sensitivity

The minimum detectable dose of human FX as calculated by 2SD from the mean of a zero standard was

established to be 0.46 ng/mL.

Specificity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Rabbit	None

protein	Cross Reactivity (%)
Factor IX	5%
Factor Xa	100%

No significant cross-reactivity observed with factor I (fibrinogen), factor II (prothrombin), factor III (tissue factor), factor V, factor VII, factor XI, factor XII, and factor XIII.

Linearity

Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
400x	105%	104%
800x	98%	101%
1600x	105%	94%

Recovery

Recovery was determined by spiking two plasma samples with different FX concentrations.

Sample	Unspiked Sample (ng/mL)	Spiked Sample (ng/mL)	Expected	Observed	Recovery (%)
1	6.2	5.0	11.2	10.8	96%
		15.0	21.2	20.1	95%
		25.0	31.2	28.3	91%
2	12.3	5.0	17.3	19.4	112%
		15.0	27.3	26.8	98%
		25.0	37.3	35.6	95%
Average Recovery (%)					98%

Precautions

1. This product is for Research Use Only and is not intended for used in diagnostic procedures.
2. Prepare all reagents (diluents buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
3. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
4. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
5. The Stop Solution is an acidic solution.
6. The kit should not be used beyond the expiration date.

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