Human Factor XII
ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

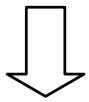
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

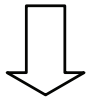
Thank you for choosing Assaypro.

Assay Summary

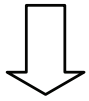
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



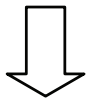
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 µl of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 30 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Factor XII ELISA Kit

Catalog No. EF1012-1
Sample Insert/Reference Only

Introduction

Human coagulation factor XII (FXII), Hageman factor, is a plasma serine protease existing in the zymogen form. Upon contact with negatively charged artificial or biologic surfaces, FXII is autoactivated into FXIIa that initiates intrinsic blood coagulation, fibrinolysis, and activation of the inflammatory kallikrein-kinin, and complement systems (1-3). FXII has 615 amino acids, weighs 80 kDa, and circulates in normal plasma at a concentration of 30 µg/ml (4, 5). It is a multidomain protein with structure similarity to EGF, single chain urokinase, and tissue plasminogen activator. In the intravascular compartment, FXII binds to endothelial cell urokinase plasminogen activator receptor, cytokeratin 1, and the complement receptor (6). FXII deficiency or blockade protects from cerebral ischemia without overtly affecting hemostasis. FXII inhibition could be a novel target for safer anticoagulation and stroke prevention without the side effect of increased bleeding (7, 8).

Principle of the Assay

The AssayMax Human Factor XII (FXII) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human factor XII in plasma, serum, milk, urine, and cell culture supernatant samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures FXII in 4 hours. A murine antibody specific for FXII has been pre-coated onto a 96-well microplate with removable strips. FXII in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for FXII, 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.

Caution and Warning

- **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.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human FXII Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against FXII.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human FXII Standard:** Human FXII in a buffered protein base (250 ng, lyophilized).
- **Biotinylated Human FXII Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against FXII (140 µl).
- **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 µl).
- **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).

Storage Condition

- 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.
- 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.
- Store Standard 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 µl, 20-200 µl, 200-1000 µl, 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 1:1000 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 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 1:1000 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.
- **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. The samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples 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 x *g* for 10 minutes. Milk dilution is suggested at 1:4 in EIA Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 FXII Standard:** Reconstitute the 250 ng of Human FXII Standard with 2.5 ml of EIA Diluent to generate a 100 ng/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 (100 ng/ml) 1:4 with EIA Diluent to produce 25, 6.25, 1.563, 0.391, 0.098, and 0.024 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 30 days.

Standard Point	Dilution	[FXII] (ng/ml)
P1	Standard (100 ng/ml)	100.0
P2	1 part P1 + 3 parts EIA Diluent	25.00
P3	1 part P2 + 3 parts EIA Diluent	6.250
P4	1 part P3 + 3 parts EIA Diluent	1.563
P5	1 part P4 + 3 parts EIA Diluent	0.391
P6	1 part P5 + 3 parts EIA Diluent	0.098
P7	1 part P6 + 3 parts EIA Diluent	0.024
P8	EIA Diluent	0.000

- **Biotinylated Human FXII 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, working standards, 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 µl of Human Factor XII 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 µl 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 machine, wash six times with 300 µl 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 µl of Biotinylated Human FXII Antibody to each well and incubate for 1 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 about 30 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 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.

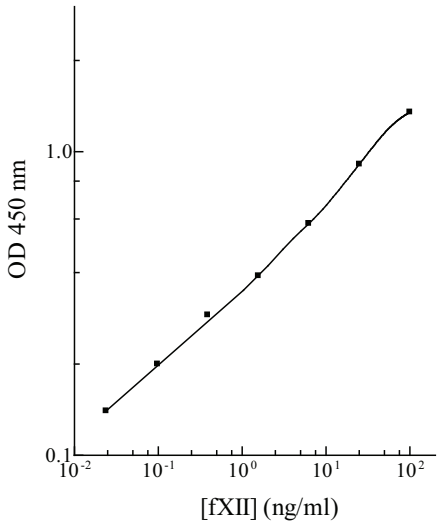
Data Analysis

- 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.

fXII Standard Curve



Performance Characteristics

- The minimum detectable level of FXII is typically ~ 0.02 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.1 % respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:500	98%	98%
1:1000	99%	100%
1:2000	103%	104%

	Average Percentage of Expected Value	
Sample Dilution	**Urine**	**Milk**
No Dilution	99%	-
1:2	97%	98%
1:4	95%	100%
1:8	-	105%

Recovery

Standard Added Value	0.098 – 25 ng/ml
Recovery %	86 – 115%
Average Recovery %	98.5%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	5%
Mouse	None
Rat	None
Swine	None
Rabbit	None
Human	100%
Protein	**% Cross Reactivity**
Human Factor XIIa	100%

Reference Values

- On average, normal human factor XII plasma level is 30 µg/ml.

References

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Version 2.1