

## Human F12 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human F12 concentrations in serum and plasma.

### General Information

Catalogue Number	KE00034
Product Name	Human F12 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.5-32 ng/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	2161
SwissProt	P00748

### Kit Components & Storage

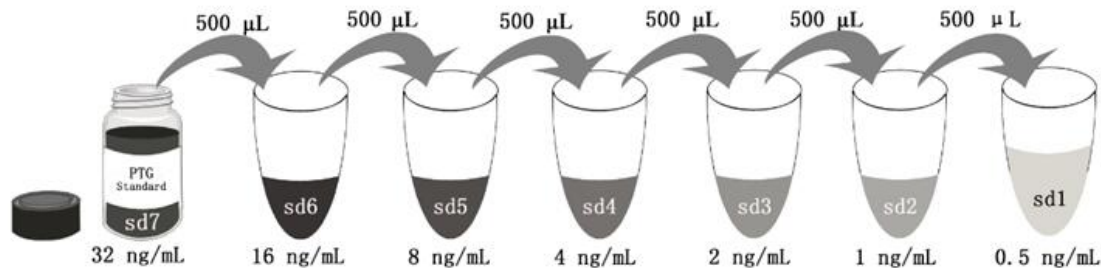
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 64 ng/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated antibody (HRP) (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 1-eg - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-eg is for protein standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

\*Add 2 mL Sample Diluent PT 1-eg in protein standard. This reconstitution gives a stock solution of 32 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-eg	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00034 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The F12 ELISA kit is to be used to detect and quantify protein levels of endogenous F12. The assay recognizes human F12. An antibody specific for F12 has been pre-coated onto the microwells. The F12 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for F12 is added to detect the captured F12 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

Factor XII (FXII, Hageman factor) is a 80kD, single chain glycoprotein that circulates in blood as an inactive zymogen. FXII plays an important role in blood coagulation, fibrinolysis, and kinin generation. When plasma comes into contact with negatively charged surfaces such as glass, kaolin, or dextran sulfate, FXII is bound to the surface and undergoes limited proteolysis by plasma kallikrein, resulting in two active enzyme forms,  $\alpha$ -FXIIa and  $\beta$ -FXIIa. Hereditary deficiencies in FXII are not associated with spontaneous or excessive bleeding. FXII inhibition also have protective role against arterial thrombosis and stroke, suggesting that FXII may be a target for antithrombotic therapy. This kit is used to quantify FXII in vivo.

## Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

## Safety Notes

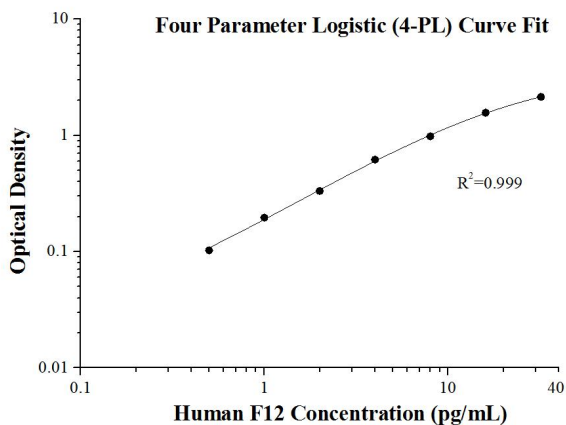
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.035 0.042	0.0385	-
0.5	0.144 0.138	0.141	0.1025
1	0.238 0.231	0.2345	0.196
2	0.385 0.356	0.3705	0.332
4	0.653 0.66	0.6565	0.618
8	1.066 0.972	1.019	0.9805
16	1.639 1.567	1.603	1.5645
32	2.168 2.19	2.179	2.1405

## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	12.18	0.77	6.40
2	20	2.90	0.19	6.70
3	20	0.55	0.03	6.00

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	12.29	1.26	10.30
2	24	2.95	0.19	6.30
3	24	0.61	0.03	5.50

## Recovery

The recovery of F12 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 94%, ranging from 73-124%

## Sample Values

Plasma samples from healthy volunteers were evaluated for F12 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human plasma (n=24)	2.30	0.24-17

## Sensitivity

The minimum detectable dose of human F12 is 0.01 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of F12 in human plasma and diluted with the appropriate **Sample Diluent PT 1-eg** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma
1:2	Average% of Expected	97
	Range (%)	93-101
1:4	Average% of Expected	96
	Range (%)	94-97
1:8	Average% of Expected	103
	Range (%)	102-105
1:16	Average% of Expected	106
	Range (%)	106-107

## References

Renne et al. Defective thrombus formation in mice lacking coagulation factor XII. J. Exp. Med. 202: 271-281, (2005).