ab289650 – Human Factor X SimpleStep ELISA®

For the quantitative measurement of Factor X in human serum, plasma (citrate), plasma (EDTA), cell culture supernatant, urine, milk, saliva, and cerebrospinal fluid.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab289650

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
Human Factor X Capture Antibody 10X	600 µL	+4°C
Human Factor X Detector Antibody 10X	600 µL	+4°C
Human Factor X Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Factor X standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 20,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 340 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	60	340	20,000	3,000
2	Standard#1	150	150	3,000	1,500
3	Standard#2	150	150	1,500	750
4	Standard#3	150	150	750	375
5	Standard#4	150	150	375	187.50
6	Standard#5	150	150	187.50	93.75
7	Standard#6	150	150	93.75	46.88
8	Blank Control	0	150	N/A	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1: 128,000 – 1: 4,000
Plasma – Citrate	1: 128,000 – 1: 4,000
Plasma – EDTA	1: 128,000 – 1: 4,000
HepG2 cell culture supernatant	1: 1,280 – 1:80
Pooled Sex Urine	≤ 50%
Milk	≤ 6.25%
Male Saliva	1.6 – 50%
CSF	1: 256 – 1: 16

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1: 4,000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1: 4,000 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1: 80 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Cerebrospinal Fluid (CSF) Dilute cerebrospinal fluid at least 1: 16 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1: 2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1: 2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.
Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1: 16 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

   Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Factor X.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), cell culture supernatant, saliva, and CSF.

Spiked protein experiments were used to validate urine and milk samples.

Cell and tissue extract samples have not been tested with this kit.

This kit is incompatible with plasma (heparin) samples.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1: 8,000 serum samples of various species, interpolating the Factor X protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the Factor X protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

7% cross-reactivity with monkey serum was observed.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.073	0.069	0.071
46.88	0.117	0.112	0.114
93.75	0.156	0.164	0.160
187.50	0.251	0.265	0.258
375	0.447	0.466	0.457
750	0.793	0.852	0.822
1,500	1.608	1.639	1.623
3,000	2.979	3.104	3.041

Table 1. Example of human Factor X standard curve in Sample Diluent NS. The Factor X standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 14.63 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=15) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Factor X were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Serum (1: 64,000)	99	97 – 101
Plasma – Citrate (1: 64,000)	104	99 – 112
Plasma – EDTA (1: 64,000)	90	87 – 93
HepG2 Cell Culture Supernatant (1: 300)	107	98 – 122
Urine (50%)	100	96 – 107
Milk (6.25%)	92	89 – 95
Saliva (5%)	98	97 – 102
CSF (1%)	96	95 – 97

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Factor X was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1:4,000 Human Serum	1:4,000 Human Plasma (Citrate)	1:4,000 Human Plasma (EDTA)
Undiluted	pg/mL	2,417	2,661	2,647
	% Expected value	100	100	100
2	pg/mL	1,225	1,377	1,383
	% Expected value	101	103	104
4	pg/mL	613	706	656
	% Expected value	101	106	99
8	pg/mL	322	365	323
	% Expected value	107	110	98
16	pg/mL	158	179	164
	% Expected value	105	107	99

Dilution Factor	Interpolated value	1:80 HepG2 supernatant	50% Human Saliva	1:16 Human CSF	6.25% Human Milk
Undiluted	pg/mL	795	2,629	1,589	191
	% Expected value	100	100	100	100
2	pg/mL	352	1,343	844	112
	% Expected value	89	102	106	118
4	pg/mL	197	717	449	NL
	% Expected value	99	109	113	NL
8	pg/mL	117	387	229	ND
	% Expected value	118	118	115	ND
16	pg/mL	58	192	117	ND
	% Expected value	118	117	118	ND

NL – Non-Linear
ND – Not Detected – below product dynamic range

Recombinant Factor X was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Pooled Sex Urine	6.25% Human Milk
Undiluted	pg/mL	1,645	1,350
	% Expected value	100	100
2	pg/mL	861	732
	% Expected value	105	108
4	pg/mL	438	377
	% Expected value	106	112
8	pg/mL	231	201
	% Expected value	112	119
16	pg/mL	108	92
	% Expected value	105	110

Precision

Mean coefficient of variations of interpolated values of Factor X from two concentrations of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	6.7	7.2

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus

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