ab287172 – Human Factor XIIIa SimpleStep ELISA® Kit

For the quantitative measurement of Factor XIIIa in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab287172

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 XIIIa Capture Antibody 10X	600 µL	+4°C
Human Factor XIIIa Detector Antibody 10X	600 µL	+4°C
Human Factor XIIIa Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BR	6 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Denaturant	500 µL	+4°C
Sample Diluent NS	100 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
The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

10X Denaturant: Prepare 10X Denaturant by diluting Denaturant stock to 10X with deionized water. To make 200 µL 10X Denaturant combine 111 µL Denaturant Stock and 89µL deionized water. Mix thoroughly and gently.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 5 mL Sample Diluent NS + 1X Enhancer, combine 4.9 mL Sample Diluent NS and 100 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

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 4BR. 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 4BR. 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. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the Factor XIIa standard by adding that volume of Sample Diluent NS + 1X Enhancer indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Factor XIIa standard by adding 500 µL Sample Diluent NS + 1X Enhancer. Hold at room temperature for 10 minutes and mix gently. This is the 240 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 350 µL of Sample Diluent NS + 1X Enhancer into tube number 1 and 150 µL of Sample Diluent NS + 1X Enhancer 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. (ng/mL)	Final Conc. (ng/mL)
1	**Stock Standard**	50	350	240	30
2	Standard#1	150	150	30	15
3	Standard#2	150	150	15	7.5
4	Standard#3	150	150	7.5	3.75
5	Standard#4	150	150	3.75	1.88
6	Standard#5	150	150	1.88	0.94
7	Standard#6	150	150	0.94	0.47
8	Blank Control	0	150	N/A	N/A

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:3200 – 1:200
Plasma - Heparin	1:6400 – 1:400
Plasma - EDTA	1:6400 – 1:400
Plasma - Citrate	1:6400 – 1:400
Serum free cell culture media	≤10%

Treat serum and plasma samples with 10X Denaturant prior to assaying.

1) Add 10µL of 10X Denaturant to 20 µL of undiluted serum or plasma. Incubate at room temperature for 5 minutes.
2) Add 70 µL Sample Diluent NS (without Enhancer) to treated serum or plasma. Mix gently and Incubate at room temperature for 10 minutes.
3) Add 1900 µL Sample Diluent NS (without Enhancer) to treated serum or plasma. Mix gently. The serum or plasma is now at 1:100 dilution.
4) Make additional dilutions with Sample Diluent NS + 1X Enhancer.

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. Treat samples with 10X Denaturant. Dilute samples at least 1:200 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted or treated serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Treat samples with 10X Denaturant. Dilute samples at least 1:400 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted or treated plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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 1:10 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles. Use serum free media, due to bovine serum cross reactivity.

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

Version 1 | 2021-09-03

ab287172 – Human Factor XIIIa SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human Factor XIIIa.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), and plasma (citrate).

Spiked protein experiments were used to validate cell culture supernatant sample types.

Saliva, urine, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
30 ng/mL of recombinant Human Fibrinogen Alpha, Human Fibrinogen Beta, and Human Fibrinogen Gamma were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
30 ng/mL of recombinant Human Fibrinogen Alpha, Human Fibrinogen Beta, and Human Fibrinogen Gamma were tested for interference with 7.5 ng/mL of recombinant Human Factor XIIIa. No interference was observed.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:200 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

100% Reactivity was observed for the following species: Human, Monkey, Mouse, Rat, Dog, Cow

Other species reactivity not determined.

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 (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0.000	0.077	0.081	0.079
0.469	0.165	0.145	0.155
0.938	0.224	0.208	0.216
1.875	0.331	0.314	0.322
3.750	0.545	0.508	0.526
7.500	0.968	0.957	0.962
15.000	1.657	1.579	1.618
30.000	3.121	2.981	3.051

Table 1. Example of human Factor XIIIa standard curve in Sample Diluent NS + 1X Enhancer. The Factor XIIIa 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 0.092 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery
3 concentrations of Factor XIIIa 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 (%)
1:800 Serum	85	81 - 88
1:1000 Plasma - Heparin	89	87 - 93
1:1000 Plasma - Citrate	89	88 - 89
1:1000 Plasma - EDTA	91	87 - 95
10% Cell culture media	122	115 - 126

*Media is serum free RPMI 1640.

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 XIIIa was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	1:200 Human Serum	1:400 Human Plasma (Citrate)	1:400 Human Plasma (EDTA)	1:400 Human Plasma (Heparin)
Undiluted	ng/mL	21.75	29.81	24.32	24.01
	% Expected value	100	100	100	100
2	ng/mL	10.58	15.84	13.04	12.53
	% Expected value	97	106	107	104
4	ng/mL	5.69	7.89	6.80	6.19
	% Expected value	105	106	112	103
8	ng/mL	2.76	3.67	3.50	2.90
	% Expected value	102	98	115	97
16	ng/mL	1.29	1.70	1.52	1.36
	% Expected value	95	91	100	91

Recombinant Factor XIIIa was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	12.5% Cell culture media
Undiluted	ng/mL	3.89
	% Expected value	100
2	ng/mL	1.72
	% Expected value	88
4	ng/mL	0.79
	% Expected value	81
8	ng/mL	0.39
	% Expected value	81

Precision

Mean coefficient of variations of interpolated values of Factor XIIIa from three concentrations of human serum 1: 400 within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.4	4.5

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

Version 1 | 2021-09-03

ab287172 – Human Factor XIIIa SimpleStep ELISA® Kit

Text templates for figure legends:

Standard Curve.

Short

Example of human Factor XIIIa standard curve in Sample Diluent NS + 1X Enhancer.

Long

Example of human Factor XIIIa standard curve. Background-subtracted data values (mean +/- SD) are graphed.

Linearity of Dilution.

Short

Interpolated concentrations of human Factor XIIIa in serum, plasma (citrate), plasma (EDTA), and plasma(heparin).

Long

Interpolated concentration of native Factor XIIIa was measured in duplicate at different sample concentrations. Undiluted samples are as follows: serum 1:200, plasma (citrate) 1:400, plasma (EDTA) 1:400, and plasma (heparin) 1:400. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Booklet version information.

v8.02. 23July2021 SADet
removed redundant text and corrected typos

v8.01. 21July2021 SADet
Added content control text to populate rest of document.
Added figure legend template text.
Formatting guidelines:

- At submission: all highlighting removed
- At submission, all extraneous text removed
- Goal is sections 1,2 are each 1 double-sided page. If the content can't be condensed to 2 pages on your best judgement, it is OK to be longer.
- Tables should not be split across a page or half-page

v8.0. 19July2021 SADet
This is the new shortened protocol booklet starting NPD July 2021 with the following aims: clearer for customer and Sci Supp (fewer pages, re-organized), less paper (logistics & customer printing), ease of NPD submission (currently modest; goal is this streamlined booklet to be integrated to future ELN for real booklet auto-generation).

- Template is here: O:\R&D\Share\New Product\Templates\SSE_Templates\SSE Booklets
    - The template isn't smart yet – the autopopulation still needs to be added,
    - Template similar to current booklet template – built to remove what isn't needed.
    - Highlighting code: requires update. Remove if not needed. Clarifying instruction. Enter here for autofill in document.
- Orientation:
    - **Section 1: How to run the assay**. This is the format of the original post-publication conversions that we reviewed previously. Materials, Prep, Protocol. No product data.
        - One notable change – std curve prep: a table replaces the graphic. This should be much easier to populate, modify.
    - **Section 2: "additional information"**: contains specificity, reactivity, product data tables. Product data tables in this section will not be added as images to the product page (this is an improvement over the last iteration)
        - Specificity is top of page (along with cross reactivity and species reactivity). Explicit about what samples have native validation and which samples are spike validated.
    - **Section 3. Figure legend templates**.
        - Text for the figure legends that will be on the product page. The product webpage page is the only place the figure images show up. This is where we will provide the text for NPI to populate adminsite.
        - Current plan is that you don't need to paste the figure into the word doc. That means only .jpg image file needed for product submission.
        - *This is the current weak part of this process – Goal to improve this part after July submission trial. Better to provide Cade with more accessible text – less copy/paste for him & simple completion for NPD.*
    - **Section 4. Version information, explanation**
- What happens with this submitted Word file?
    - Sections 1, 2.
        - Printed and shipped with product – this probable to stop in near future. Customer can print if needed.
        - PDF hyperlink on product page.
        - Word file is on adminsite for ease of future updates
    - Section 3. None of the above – used for populating adminsite but isn't printed by logistics and does not show up on product page.
    - Section 4. For internal reference only. Not on adminsite or product page.
- *July 2021 NPD: Fully expect this template to need revision and correction and improvement. Everyone's feedback appreciated to make improvements.*