ab241383 – Human Fibrinogen SimpleStep ELISA® Kit

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

For overview, typical data and additional information please visit: www.abcam.com/ab241383

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pg 11.

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 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human Fibrinogen Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Fibrinogen Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Fibrinogen Lyophilized Purified Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	10 x 96 wells	+4°C
Plate Seal	1	10	+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 Fibrinogen 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 100,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 368 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**	32	368	100,000	8,000
2	Standard#1	150	150	8,000	4,000
3	Standard#2	150	150	4,000	2,000
4	Standard#3	150	150	2,000	1,000
5	Standard#4	150	150	1,000	500
6	Standard#5	150	150	500	250
7	Standard#6	150	150	250	125
8	Blank Control	0	150	N/A	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	$1:10^4$ – 1:670
Plasma – Citrate	$1:8x10^6$ – $1:5x10^5$
Plasma – EDTA	$1:8x10^6$ – $1:5x10^5$
Plasma – Heparin	$1:8x10^6$ – $1:5x10^5$
Urine	3.75 – 60%
Saliva	1:2,000 – 1:133
Milk	1: 2,000 – 1:133
HepG2 Cell Culture Supernatant	1:8,000 – 1:500

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 1:670 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, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least $1:5x10^5$ 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.

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 1:500 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 1:1.7 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 1:133 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 1:133 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Note: Due to the high dilutions required for plasma samples, we recommend initially diluting your samples in 1X Wash Buffer and then performing the final dilution in Sample Diluent NS. As an example, the table below demonstrates the steps suggested to generate a final sample dilution of $1:5x10^5$:

Tube #	Sample to dilute	Volume of Sample (µL)	Volume of 1X Wash Buffer (µL)	Volume of NS (µL)	Starting Conc.	Final Conc.
1	Neat Plasma	30	270	-	Neat	1:10
2	Tube #1	30	270	-	1:10	1:100
3	Tube #2	30	-	270	1:100	$1:1x10^3$
4	Tube #3	30	-	270	$1:1x10^3$	$1:1x10^4$
5	Tube #4	30	-	270	$1:1x10^4$	$1:1x10^5$
6	Tube #5	30		120	$1:1x10^5$	$1:5x10^5$

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 7 – 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 4b | 22/02/224

ab241383 – Human Fibrinogen SimpleStep ELISA® Kit

Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Fibrinogen.

The standard protein in this kit is full length human Fibrinogen.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, saliva, urine, and milk sample types.

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

CROSS REACTIVITY

The following recombinant proteins were prepared at 50 ng/mL and 8,000 pg/mL and assayed for cross reactivity and no cross reactivity was observed.

- Human Fibrinogen alpha
- Human Fibrinogen beta
- Human Fibrinogen gamma
- Human Factor XII
- Human Plasmin
- Human Thrombin
- Rat Fibrinogen

The following recombinant proteins were prepared at 50 ng/mL and 8,000 pg/mL and assayed for cross reactivity.

- Human D-Dimer: 27% cross-reactivity

INTERFERENCE

The following recombinant proteins were prepared at 50 ng/mL and 8,000 pg/mL and assayed for interference and no interference was observed.

- Human Fibrinogen alpha
- Human Fibrinogen beta
- Human Fibrinogen gamma
- Human D-Dimer
- Human Factor XII
- Human Plasmin
- Human Thrombin
- Rat Fibrinogen

SPECIES REACTIVITY

This kit recognizes human Fibrinogen protein.

Native purified mouse Fibrinogen was prepared at 8,000 pg/mL and assayed for cross reactivity. 100% cross reactivity was observed.

Other species reactivity was determined by measuring 1:670 diluted 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.

Reactivity < 3% was determined for the following species:

– Rat

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 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	0.116	0.114	0.115
125	0.199	0.202	0.201
250	0.285	0.287	0.286
500	0.457	0.450	0.454
1,000	0.748	0.768	0.758
2,000	1.314	1.360	1.337
4,000	2.392	2.457	2.424
8,000	3.515	3.550	3.533

Table 1. Example of human Fibrinogen standard curve in Sample Diluent NS. The Fibrinogen 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 29 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Fibrinogen 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 (%)
1:5,333 Serum	91%	90 – 94%
1:4x10^6 Plasma – Heparin	95%	95%
1:4x10^6 Plasma – EDTA	93%	91 – 94%
1:4x10^6 Plasma – Citrate	94%	91 – 96%
7.5% Urine	100%	99 – 100%
1:1,067 Saliva	101%	98 – 104%
1:1,067 Milk	103%	102 – 105%
1:2,000 HepG2 Cell Culture Supernatant	94%	93 – 95%

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 Fibrinogen 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	1:670 Human Serum	1:5x10^5 Human Plasma (Citrate)	1:5x10^5 Human Plasma (EDTA)	1:5x10^5 Human Plasma (Heparin)
Undiluted	pg/mL	5,054	5,693	5,787	5,035
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	2,473	2,787	2,786	2,510
	% Expected value	**98**	**98**	**96**	**100**
4	pg/mL	1,305	1,411	1,473	1,322
	% Expected value	**103**	**99**	**102**	**105**
8	pg/mL	656	726	758	682
	% Expected value	**104**	**102**	**105**	**108**
16	pg/mL	336	371	395	353
	% Expected value	**106**	**104**	**109**	**112**

Native Fibrinogen 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	60% Human Urine	1:133 Human Saliva	1:133 Human Milk	1:500 HepG2 Cell Culture Supernatant
Undiluted	pg/mL	3,36	7,458	1,535	2,610
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	1,749	3,389	771	1,371
	% Expected value	**105**	**91**	**101**	**105**
4	pg/mL	920	1,689	401	701
	% Expected value	**110**	**91**	**105**	**107**
8	pg/mL	462	875	190	351
	% Expected value	**111**	**94**	**99**	**108**
16	pg/mL	223	441	80	159
	% Expected value	**107**	**95**	**84**	**98**

Precision

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

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.4	11.7

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Version 4b | 22/02/224

DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human Fibrinogen Capture Antibody 10X	600 µL	+4°C
Human Fibrinogen Detector Antibody 10X	600 µL	+4°C
Human Fibrinogen Lyophilized Purified Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	250 mL	+4°C
Wash Buffer PT 10X	2 x 20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 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 in a 384-well plate.
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).
Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 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).

5. Reconstitute the Fibrinogen 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 100,000 pg/mL **Stock Standard** Solution.
6. Label eight tubes, Standards 1– 8.
7. Add 368 µL of Sample Diluent NS into tube number 1 and 75 µL of Sample Diluent NS into numbers 2-8.
8. 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**	32	368	100,000	8,000
2	Standard#1	75	75	8,000	4,000
3	Standard#2	75	75	4,000	2,000
4	Standard#3	75	75	2,000	1,000
5	Standard#4	75	75	1,000	500
6	Standard#5	75	75	500	250
7	Standard#6	75	75	250	125
8	Blank Control	0	75	N/A	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
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 for 384-well plate format

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. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 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.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 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.
7. Add 25 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. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: 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 25 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:
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Version 4b | 22/02/224