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Mouse Fibrinogen SimpleStep ELISA® Kit

For the quantitative measurement of Fibrinogen in mouse serum, plasma, urine, cell culture supernatant, and cell and tissue extract samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Fibrinogen *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Fibrinogen protein in mouse serum, plasma, urine, cell culture supernatant, and cell and tissue extract samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Fibrinogen is a heterohexamer of molecular mass 340 kDa, made up of two sets of alpha, beta, gamma polypeptide chains, and synthesized in the parenchymal cell of the hepatocyte and in the megakaryocyte. Fibrinogen plays a major role in hemostasis as one of the primary component of coagulation, and both elevated and decreased levels have clinical significance. The conversion of fibrinogen to fibrin is triggered by thrombin, which cleaves fibrinopeptides A and B from alpha and beta chains, and thus exposes the N-terminal polymerization sites responsible for the formation of the soft clot. Upon cleavage by thrombin, Fibrinogen self-assembles to yield a fibrin clot matrix that subsequently is crosslinked by factor XIIIa to form an insoluble network. Fibrinogen also binds to the platelet glycoprotein IIb and IIIa receptor so as to form bridges between platelets, thus facilitating aggregation. Elevated plasma Fibrinogen has been identified as an independent risk factor for coronary atherosclerosis and ischemic heart disease. Individuals with congenital absence of Fibrinogen, termed a fibrinogenemia, have prolonged bleeding times. Defects in Fibrinogen,

alpha are a cause of amyloidosis type 8 (AMYL8) also known as systemic non-neuropathic amyloidosis or Ostertag-type amyloidosis.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
10X Mouse Fibrinogen Capture Antibody	600 µL	+4°C
10X Mouse Fibrinogen Detector Antibody	600 µL	+4°C
Mouse Fibrinogen Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BR	6 mL	+4°C
10X Wash Buffer PT	20 mL	+4°C
5X Cell Extraction Buffer PTR	10 mL	+4°C
50X Cell Extraction Enhancer Solution	1 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- 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).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.

- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. 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.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL 5X Cell Extraction Buffer PTR. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 1X Wash Buffer PT

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

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent. 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 5BR. Mix thoroughly and gently.

10. 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).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the mouse Fibrinogen standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the mouse Fibrinogen standard by adding 500 µL Diluent.
serum, plasma, urine and cell culture supernatant samples measurements, reconstitute the mouse Fibrinogen protein standard by adding Sample Diluent NS.
For cell and tissue extract samples measurements, reconstitute the mouse Fibrinogen protein standard by adding 1X Cell Extraction Buffer PTR.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 255 µL of appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2 8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Mouse Fibinogen (ng/mL)
1	45 µL Stock	255	14,000
2	150 µL Standard #1	150	7,000
3	150 µL Standard #2	150	3,500
4	150 µL Standard #3	150	1,750
5	150 µL Standard #4	150	875
6	150 µL Standard #5	150	437.5
7	150 µL Standard #6	150	218.75
8 (Blank)	N/A	300	0

11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Mouse Plasma - Heparin	1:6400 – 1:400
Mouse Plasma - EDTA	1:3200 – 1:100
Mouse Plasma - Citrate	1:1280 - 1:10
Mouse Serum	1 – 50%
3T3L1 Cell Culture Supernatant (differentiated) 10 Days	1.5 – 25%
Liver Cell Culture Supernatant 5 Days	6.25 – 100%
Lung Cell Culture Supernatant 6 Days	1.5 – 50%
Mouse Liver Extract	9.38 – 300 µg/mL

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma (heparin) samples at least 400 fold into Sample Diluent NS and assay. Dilute plasma (EDTA) samples at least 100 fold into Sample Diluent NS and assay. Dilute plasma (Citrate) samples at least 10 fold 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.

11.2 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 serum samples at least 2 fold into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 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 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 Preparation of extracts from cell pellets:

11.5.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.5.2 Rinse cells twice with PBS.

11.5.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.5.4 Incubate on ice for 20 minutes.

11.5.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.6 Transfer the supernatants into clean tubes and discard the pellets.

11.5.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.6 Preparation of extracts from adherent cells by direct lysis (alternative protocol)

11.6.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.6.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.6.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.7 Preparation of extracts from tissue homogenates

11.7.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.7.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.7.3 Incubate on ice for 20 minutes.

11.7.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.7.5 Transfer the supernatants into clean tubes and discard the pellets.

11.7.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.7.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. 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.

13. 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.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 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.

13.3 Add 50 µL of all samples and standards to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 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.

13.7 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.

13.8 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.

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 minutes
Interval:	20 sec - 1 minute
Shaking:	Shake between readings

Δ **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 450nm.

13.9 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 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.

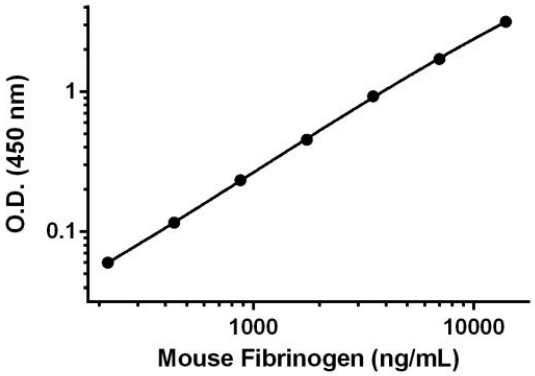
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 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.

14.4 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.

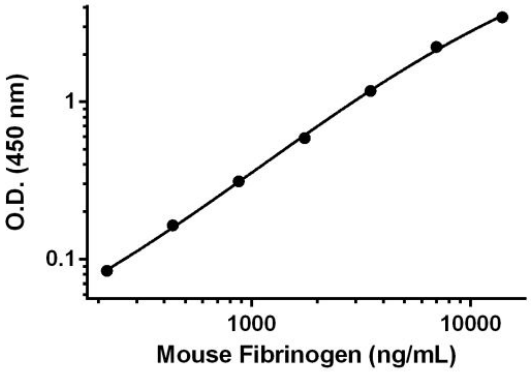
15. Typical Data

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



Standard Curve Measurements			
Conc.	O.D. 450 nm		Mean
(ng/mL)	1	2	O.D.
0	0.059	0.060	0.060
218.75	0.121	0.119	0.120
437.5	0.174	0.176	0.175
875	0.295	0.291	0.293
1,750	0.511	0.515	0.513
3,500	0.975	1.002	0.988
7,000	1.673	1.881	1.777
14,000	3.160	3.292	3.226

Figure 1. Example of mouse Fibrinogen standard curve in Sample Diluent NS. The Fibrinogen standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc.	O.D. 450 nm		Mean
(ng/mL)	1	2	O.D.
0	0.076	0.078	0.077
218.75	0.161	0.162	0.161
437.5	0.240	0.241	0.241
875	0.387	0.390	0.389
1,750	0.664	0.668	0.666
3,500	1.238	1.265	1.252
7,000	2.294	2.325	2.310
14,000	3.497	3.559	3.528

Figure 2. Example of mouse Fibrinogen standard curve in 1X Cell Extraction Buffer PTR. The Fibrinogen standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
1 X Cell Extraction Buffer PTR	16	41 ng/mL
Sample Diluent NS	17	38 ng/mL

RECOVERY –

Three concentrations of mouse Fibrinogen native purified protein 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 (%)
150 µg/mL Mouse Liver Extract	88	86 - 90
25% Mouse Serum	110	101 - 117
2% Mouse Plasma Citrate	103	98 - 108
1% Mouse Plasma EDTA	106	98 - 111
0.25% Mouse Plasma Heparin	109	105 – 115
50% RPMI cell culture media containing 10% bovine serum	118	112 - 124
5% Mouse Urine	102	100 - 104

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	50% Mouse Serum	10% Mouse Plasma (Citrate)	1% Mouse Plasma (EDTA)	1:400 Mouse Plasma (Heparin)
Undiluted	µg/mL	3.222	15.39	2.524	1.135
	% Expected value	**100**	**100**	**100**	**100**
2	µg/mL	1.792	8.354	1.240	0.529
	% Expected value	**111**	**109**	**98**	**93**
4	µg/mL	0.872	3.562	0.580	0.248
	% Expected value	**108**	**93**	**92**	**87**
8	µg/mL	0.444	1.707	0.271	0.151
	% Expected value	**110**	**89**	**86**	**107**
16	µg/mL	0.192	0.840	0.131	0.068
	% Expected value	**96**	**87**	**83**	**97**

Native Fibrinogen was measured in the following biological samples in a 2-fold dilution series. Supernatant (SN) sample dilutions are made in Sample Diluent NS and extract sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	25% 3T3L1 SN	50% Mouse Lung SN	100% Mouse Liver SN	300 µg/mL Mouse Liver Extract
Undiluted	ng/mL	821.5	12509	2952	2081
	% Expected value	**100**	**100**	**100**	**100**
2	ng/mL	408.4	6201	1629	1037
	% Expected value	**99**	**99**	**110**	**100**
4	ng/mL	187.8	2897	788.4	560.8
	% Expected value	**91**	**93**	**107**	**108**
8	ng/mL	97.02	1445	374.2	285.3
	% Expected value	**94**	**92**	**101**	**110**
16	ng/mL	47.04	714.1	211.0	150.9
	% Expected value	**92**	**91**	**114**	**116**

Mouse Fibrinogen native purified protein was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	5%Mouse Urine
Undiluted	µg/mL	15.40
	% Expected value	**100**
2	µg/mL	8.001
	% Expected value	**104**
4	µg/mL	3.741
	% Expected value	**97**
8	µg/mL	1.699
	% Expected value	**88**
16	µg/mL	0.772
	% Expected value	**80**

PRECISION –

Mean coefficient of variations of interpolated values of Fibrinogen in 3 concentrations of mouse plasma (EDTA) within the working range of the assay.

	Intra-assay	Inter-Assay
n=	3	5
CV (%)	3	2

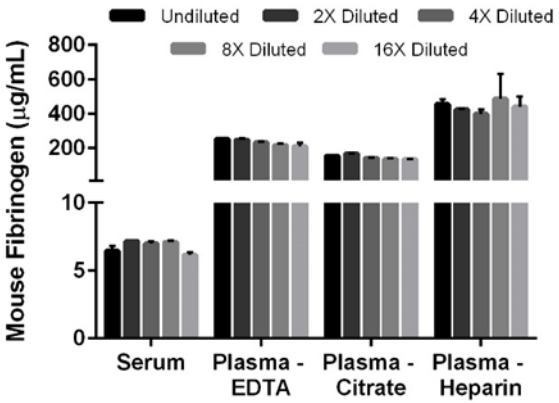


Figure 3. Interpolated concentrations of native Fibrinogen in mouse serum and plasma samples. The concentrations of Fibrinogen were measured in duplicates, interpolated from the Fibrinogen standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 50%, plasma (EDTA) 1%, plasma (citrate) 10% and plasma (heparin) 1:400. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibrinogen concentration was determined to be 6.8 µg/mL in neat serum, 232 µg/mL in neat plasma (EDTA), 146 µg/mL in neat plasma (citrate) and 440 µg/mL in neat plasma (heparin).

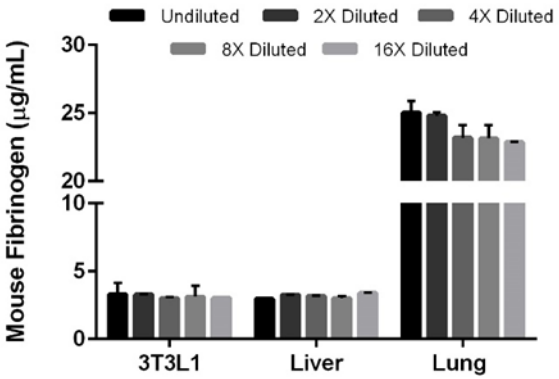


Figure 4. Interpolated concentrations of native Fibrinogen in mouse cell culture supernatant samples. The concentrations of Fibrinogen were measured in duplicates, interpolated from the Fibrinogen standard curves and corrected for sample dilution. Undiluted samples are as follows: 3T3L1 (differentiated, 10 days) 25%, liver (5 days) 100% and lung (6 days) 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibrinogen concentration was determined to be 3.1 µg/mL in neat 3T3L1, 3.1 µg/mL in neat liver and 24 µg/mL in neat lung supernatant samples.

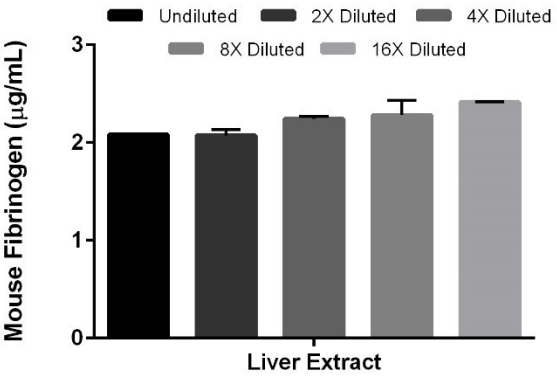


Figure 5. Interpolated concentrations of native Fibrinogen in mouse liver cell tissue extract based on a 300 µg/mL extract load. The concentrations of Fibrinogen were measured in duplicate and interpolated from the Fibrinogen standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Fibrinogen concentration was determined to be 2.2 µg/mL in mouse liver cell tissue extract.

17. Assay Specificity

This kit recognizes both native and recombinant mouse Fibrinogen protein in serum, plasma, urine, and cell culture supernatant, cell and tissue extract samples only.

Milk and saliva samples have not been tested with this kit.

18. Species Reactivity

This kit recognizes mouse Fibrinogen protein.

Other species reactivity was determined by measuring a 14 µg/mL load of rat and human natural purified Fibrinogen protein, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the mouse Fibrinogen protein assayed at the same dilution.

Level of reactivity:

Species	% Cross-reactivity
Rat	95
Human	20

Taking into consideration the % cross-reactivity, mean Fibrinogen concentration was determined to be 1,063 µg/mL in neat human plasma (citrate) and 303 µg/mL in neat rat plasma (citrate).

The kit is also reactive with cow Fibrinogen, the exact % cross-reactivity was not determined.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hours standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)