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ab222507 Human Antithrombin III / ATIII SimpleStep ELISA® Kit

For the quantitative measurement of human Antithrombin III in serum, plasma, urine, milk, and cell culture supernatant samples.

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

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1. Overview

Antithrombin III *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of human Antithrombin III protein in serum, plasma, urine, milk, and cell culture supernatant 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.

Antithrombin-III (ATIII), also known as Serpin C1, is a 58 kDa extracellular glycoprotein belonging to the Serpin serine protease inhibitor superfamily. Antithrombin III functions primarily as a blood anticoagulant but also modulates inflammatory cytokine expression. Human Antithrombin-III is synthesized in the liver and is secreted as a 432 amino acid (aa) mature protein. Most circulating Antithrombin-III (90-95%) is the fully glycosylated α-isoform while the partially glycosylated β-isoform constitutes 5-10%. The biological activity of Antithrombin-III is inherently low and is enhanced up to 1000-fold in the presence of heparin or heparin-like glycosaminoglycans. The β-isoform of Antithrombin-III has a higher affinity for heparin. Mature human Antithrombin-III shares 90% sequence homology with mature mouse Antithrombin-III. Human Antithrombin-III inactivates thrombin and other blood coagulation factors. Human adult Antithrombin-III levels in plasma are relatively constant. However, reduction of Antithrombin-III is observed in individuals with diabetes or sepsis. Neonates also exhibit low levels of Serpin C1 in plasma.

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 handle 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.

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
Human Antithrombin III Capture Antibody 10X	600 µL	+4°C
Human Antithrombin III Detector Antibody 10X	600 µL	+4°C
Human Antithrombin III Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BC	6 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Denaturant	500 µL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Anti-tag coated 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

- 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 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.
- 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 Sample Diluent NS + Enhancer:

Prepare Sample Diluent NS plus Enhancer by diluting Cell Extraction Enhancer Solution 50X to 1X with Sample Diluent NS. To make 10 mL Sample Diluent NS plus Enhancer, combine 9.8 mL of Sample Diluent NS and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently for five minutes.

9.2 5X Denaturant (For urine, milk or cell culture supernatant samples only):

To prepare 120 µL of 5X Denaturant, combine 15 µL of Denaturant with 105 µL of water. Mix thoroughly and gently.

9.3 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.

9.4 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BC. 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 5BC. 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).

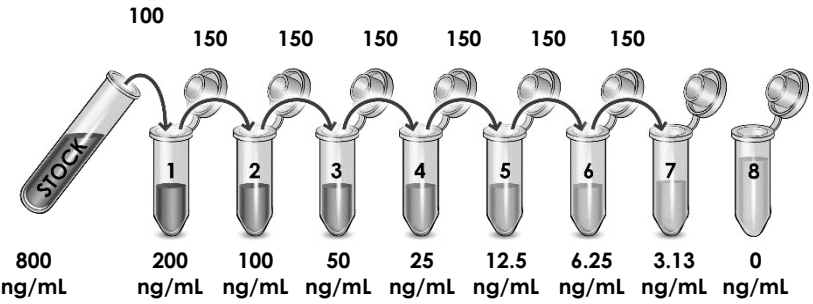
Δ **Note: The Antithrombin III standard should be stored on ice and used within 30 minutes of reconstitution.**

10.1 **IMPORTANT**: If the protein standard vial has a volume identified on the label, reconstitute the human Antithrombin III standard by adding that volume of Sample Diluent NS plus Enhancer indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the human Antithrombin III standard by adding 1,000 µL Sample Diluent NS plus Enhancer. Hold at room temperature for 10 minutes and mix gently. This is the 800 ng/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 300 µL Sample Diluent NS plus Enhancer into tube number 1 and 150 µL of Sample Diluent NS plus Enhancer 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:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Human Plasma - Heparin	1:32,000 – 1:4,000
Human Plasma - EDTA	1:32,000 – 1:4,000
Human Plasma - Citrate	1:64,000 – 1:8,000
Human Serum	1:32,000 – 1:4,000
Human Urine	1:128 – 1:16
Human Milk	1:256 – 1:32
HepG2 Cell Supernatant	1.56 – 6.25%
RPMI Culture Media	1:160 – 1:20

Δ Note: All samples must be prepared immediately prior to assaying.

11.1 Plasma: Samples require pretreatment before use.

11.1.1 Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS + Enhancer and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.1.2 Before use, the plasma samples require an activation treatment with the Denaturant. The starting plasma concentration before treatment should be concentrated enough that the final concentration after neutralization will be a sufficient in assay concentration (see example below).

11.1.3 Dilute the 4000X or 8000X plasma sample with prepared Denaturant (eg for a total volume of 72 µL add 5 µL of Denaturant to 67 µL plasma). Incubate at room temperature for 10 minutes with intermittent vortexing. This step will generate a 93.06% plasma concentration.

11.1.4 Neutralize activated sample with Sample Diluent NS by adding 12 µL of the activated plasma to 288 µL Sample Diluent NS. The resulting plasma concentration is 3.72 %

11.1.5 To prepare the activated/neutralized plasma samples for assay, further dilute with Sample Diluent NS + Enhancer to desired concentration (see table below).

11.2 Serum: Samples require pretreatment before use.

11.2.1 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 into Sample Diluent NS + Enhancer and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.2.2 Before use, the serum samples require activation treatment with the Denaturant. The starting plasma concentration before treatment should be concentrated enough that the final concentration after neutralization will be a sufficient in assay concentration (see example below).

11.2.3 Dilute the 4000X serum sample with prepared Denaturant (eg for a total volume of 72 µL add 5 µL of Denaturant to 67 µL serum). Incubate at room temperature for 10 minutes with intermittent vortexing. This step will generate a 93.06% serum concentration.

11.2.4 Neutralize activated sample with Sample Diluent NS by adding 12 µL of the activated serum to 288 µL Sample Diluent NS. The resulting serum concentration is 3.72 %

11.2.5 To prepare the activated/neutralized serum samples for assay, further dilute with Sample Diluent NS + Enhancer to desired concentration (see table below).

Example of serum and plasma dilutions:

Sample	Stock Conc. (%)	Total Volume (µL)	Stock Volume (µL)	Volume Diluent	Diluent	Final Conc (%)
Human serum & plasma (EDTA & heparin)	100	72	67	5	Denaturant	93.06
	Incubate for 10 minutes with intermittent mixing					
	93.06	300	12	288	S.D NS*	3.72
	3.72	186	25	161	S.D NS + E**	0.5
	0.5	400	20	380	S.D NS + E**	0.025
Human plasma (citrate)	100	72	67	5	Denaturant	93.06
	Incubate for 10 minutes with intermittent mixing					
	93.06	300	120	288	S.D NS*	3.72
	3.72	186	25	161	S.D NS + E**	0.5
	0.5	400	20	380	S.D NS + E**	0.025

*S.D NS – Sample Diluent NS
** S.D NS + E – Sample Diluent NS + Enhancer

11.3 Cell Culture Supernatants: Samples require pretreatment before use.

11.3.1 Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants, pretreat samples as directed and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3.2 Before use, the cell culture supernatant samples require an activation treatment with the prepared 5X Denaturant. The starting cell culture supernatant concentration before treatment should be concentrated enough that the final concentration after neutralization will be a sufficient in assay concentration (see example below).

11.3.3 Dilute the 16X cell culture supernatant sample with prepared 5X Denaturant (eg for a total volume of 135 µL add 15 µL of 5X Denaturant to 120 µL cell culture supernatant). Incubate at room temperature for 10 minutes with intermittent vortexing. This step will generate an 88.89% cell culture supernatant concentration.

11.3.4 Neutralize activated sample with Sample Diluent NS by adding 81 µL of the activated cell culture supernatant to 319 µL Sample Diluent NS. The resulting cell culture supernatant concentration is 18 %

11.3.5 To prepare the activated/neutralized cell culture supernatant samples for assay, further dilute with Sample Diluent NS+ Enhancer to desired concentration (see table below).

11.4 Urine: Samples require pretreatment before use.

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

11.4.2 Before use, the urine samples require activation treatment with the prepared 5X Denaturant. The starting urine concentration before treatment should be concentrated enough that the final concentration after neutralization will be a sufficient in assay concentration (see example below).

11.4.3 Dilute the 16X urine sample with prepared 5X Denaturant (eg for a total volume of 135 µL add 15 µL of 5X denaturant to 120 µL cell culture supernatant). Incubate at room

temperature for 10 minutes with intermittent vortexing. This step will generate an 88.89% urine concentration.

11.4.4 Neutralize activation treatment with Sample Diluent NS by adding 81 µL of the activated urine to 319 µL Sample Diluent NS. The resulting urine concentration is 18 %.

11.4.5 To prepare the activated/neutralized urine samples for assay, further dilute with Sample Diluent NS + Enhancer to desired concentration (see table below).

11.5 Milk: Milk Samples require pretreatment shown below.

11.5.1 Pretreat milk samples as directed below. If required, dilute milk samples in Sample Diluent NS + Enhancer and assay

11.5.2 Before use, the milk samples require activation treatment with the 5X Denaturant. The starting milk concentration before treatment should be concentrated enough that the final concentration after neutralization will be a sufficient in assay concentration (see example below).

11.5.3 Dilute the 32X milk sample with prepared 5X Denaturant (eg for a total volume of 135 µL add 15 µL of 5X Denaturant to 120 µL milk). Incubate at room temperature for 10 minutes with intermittent vortexing. This step will generate a 88.89% milk concentration.

11.5.4 Neutralize activation treatment with Sample Diluent NS by adding 81 µL of the activated milk to 319 µL Sample Diluent NS. The resulting milk concentration is 18 %

11.5.5 To prepare the activated/neutralized milk samples for assay, further dilute with Sample Diluent NS + Enhancer to desired concentration (see table below).

Example of cell culture supernatant, milk and urine dilutions:

Sample	Stock Conc. (%)	Total Volume (µL)	Stock Volume (µL)	Volume Diluent	Diluent	Final Conc (%)
Cell Supernatant	100	135	120	15	5X Denaturant	88.89
	Incubate for 10 minutes with intermittent mixing					
	88.89	400	81	319	S.D NS*	18
	18	432	150	282	S.D NS + E	6.25
Milk	100	135	120	15	5X Denaturant	88.89
	Incubate for 10 minutes with intermittent mixing					
	88.89	400	81	319	S.D NS*	18
	18	432	75	357	S.D NS + E	3.125
Urine	100	135	120	15	5X Denaturant	88.89
	Incubate for 10 minutes with intermittent mixing					
	88.89	400	81	319	S.D NS*	18
	18	432	150	282	S.D NS + E**	6.25

*S.D NS – Sample Diluent NS
** S.D NS + E – Sample Diluent NS + Enhancer

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 sample or standard 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.

13.9 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

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 450 nm.

13.10 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.

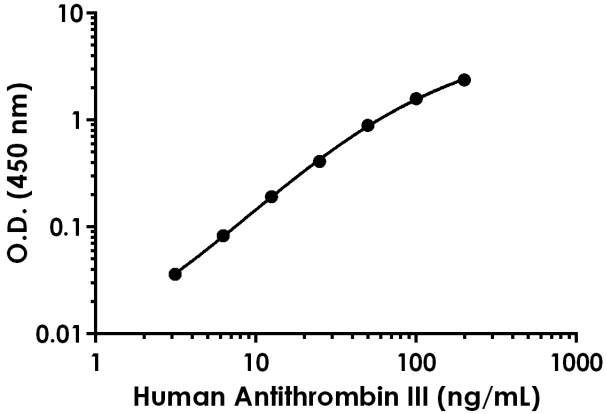
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides 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			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.049	0.054	0.051
3.13	0.087	0.088	0.087
6.25	0.134	0.134	0.134
12.5	0.245	0.242	0.244
25	0.456	0.468	0.462
50	0.958	0.932	0.945
100	1.616	1.673	1.644
200	2.428	2.440	2.434

Figure 1. Example of human Antithrombin III standard curve. The Antithrombin III 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 calculated minimal detectable dose (MDD) is 0.33 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 –

Three concentrations of human Antithrombin III 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 (%)
Human Plasma – Heparin (1:8,000)	106	97-121
Human Plasma - EDTA (1:8,000)	100	102-110
Human Plasma - Citrate (1:16,000)	107	79-106
Human Serum (1:8,000)	91	79-106
Human Urine (1:16)	87	80-93
Human Milk (1:32)	113	100-126
HepG2 Cell Supernatant (1:16)	120	106-133
RPMI Culture Media (1:20)	110	97-122

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 human Antithrombin III was measured in serum, plasma, milk, and Hep G2 supernatant samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS plus Enhancer.

Recombinant human Antithrombin III was spiked into urine and RPMI culture media and diluted in a 2-fold dilution series in Sample Diluent NS plus Enhancer.

Dilution Factor	Interpolated value	1:4,000 Human Serum	1:8,000 Human Plasma (Citrate)	1:4,000 Human Plasma (EDTA)	1:4,000 Human Plasma (Heparin)
Undiluted	ng/mL	43.88	39.67	45.81	61.19
	% Expected value	100	100	100	100
2	ng/mL	19.38	16.68	18.95	24.27
	% Expected value	88	84	83	79
4	ng/mL	9.31	8.22	9.37	12.24
	% Expected value	85	83	82	80
8	ng/mL	4.73	4.26	4.88	6.64
	% Expected value	86	86	85	87
16	ng/mL	ND	ND	ND	4.32
	% Expected value	ND	ND	ND	113

Dilution Factor	Interpolated value	6.25% Human Urine	3.13% Human Milk	6.25% Hep G2 Supernatant	5% RPMI Culture Media
Undiluted	ng/mL	41.37	26.48	15.20	40.58
	% Expected value	100	100	100	100
2	ng/mL	17.89	12.22	6.85	22.26
	% Expected value	86	92	90	110
4	ng/mL	9.05	6.09	3.04	11.57
	% Expected value	87	92	80	114
8	ng/mL	5.17	3.25	ND	6.03
	% Expected value	100	98	ND	119
16	ng/mL	NL	ND	ND	NL
	% Expected value	NL	ND	ND	NL

NL – Non-Linear, ND – Non-Detectable

PRECISION –

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

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	2.9	7.9

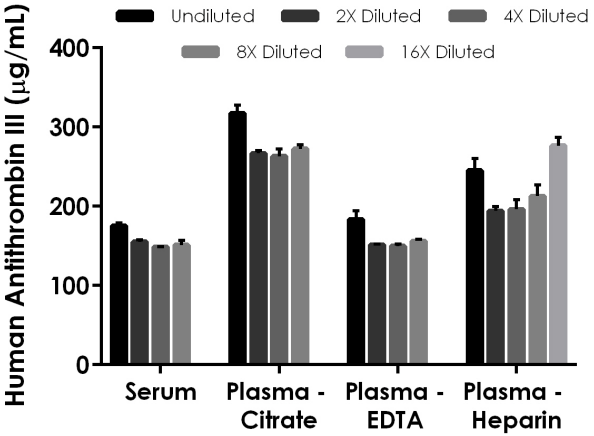


Figure 2. Interpolated concentrations of native Antithrombin III in human serum and plasma samples. The concentrations of Antithrombin III were measured in duplicates, interpolated from the Antithrombin III standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1:4,000 plasma (citrate) 1:8,000, plasma (EDTA) 1:4,000, and plasma (heparin) 1:4,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Antithrombin III concentration was determined to be 157.69 µg/mL in neat serum, 279.95 µg/mL in neat plasma (citrate), 160.27 µg/mL in neat plasma (EDTA), and 224.88 µg/mL in neat plasma (heparin).

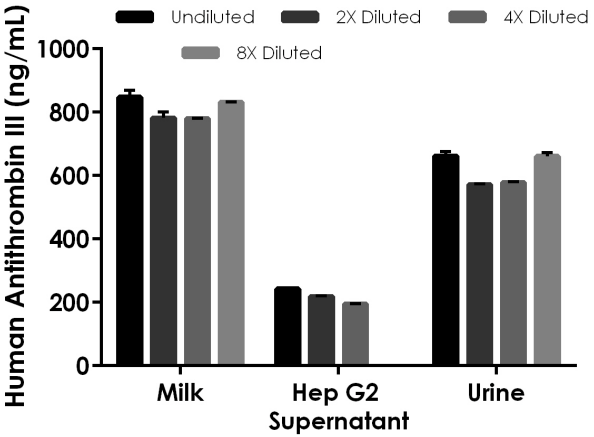


Figure 3. Interpolated concentrations of native Antithrombin III in human milk and human Hep G2 supernatant samples and spiked Antithrombin III in human urine. The concentrations of Antithrombin III were measured in duplicates, interpolated from the Antithrombin III standard curves and corrected for sample dilution. Undiluted samples are as follows: milk 3.13%, Hep G2 supernatant 6.25%, and urine 6.25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Antithrombin III concentration was determined to be 810.16 ng/mL in neat milk, 218.86 ng/mL in neat Hep G2 supernatant, and 618.60 ng/mL in neat spiked urine.

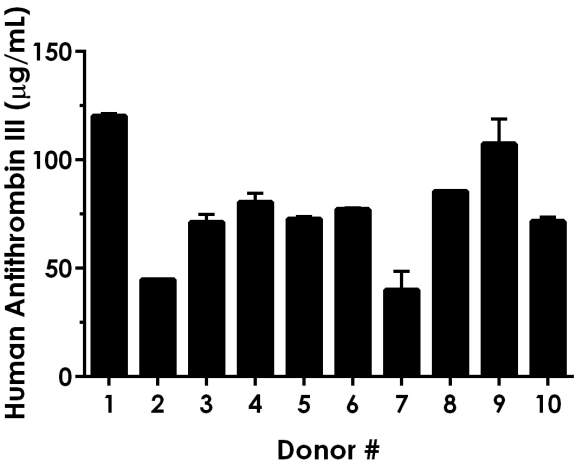


Figure 4. Serum from ten individual healthy human female donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Antithrombin III concentration was determined to be 77.20 µg/mL with a range of 34.09 – 121.12 µg/mL.

17. Assay Specificity

This kit recognizes both native human Antithrombin III protein in serum, plasma, urine, milk, and cell culture supernatant samples only.

18. Species Reactivity

This kit recognizes human Antithrombin III protein.

Other species reactivity was determined by measuring 1:4,000 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:

- Mouse
- Rat
- Cow

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 hour 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 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.

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)