ab108907 Human Thrombin-Antithrombin Complex ELISA Kit (TAT)

For the quantitative measurement of human Thrombin-Antithrombin Complex (TAT) in plasma, milk and cell culture supernatants.

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

Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Thrombin-Antithrombin Complex Microplate (12 x 8 wells)	96 wells	4°C
Thrombin-Antithrombin Complex Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Human Thrombin-Antithrombin Complex Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 6 tubes to prepare standard or sample dilution

Reagent Preparation

- Equilibrate all reagents to room temperature prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

1X Diluent N: Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. Store for up to 1 month at 4°C.

1X Wash Buffer: Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

1X Biotinylated Thrombin-Antithrombin Complex Detector Antibody:

1. The stock Biotinylated Thrombin-Antithrombin Complex Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Thrombin-Antithrombin Complex Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated Thrombin-Antithrombin Complex Antibody.
2. Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Thrombin-Antithrombin Complex Antibody to prepare a 1X Biotinylated Thrombin-Antithrombin Complex Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

No. of Wells Strips	No. of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Δ Note Any remaining solution should be frozen at -20°C.

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Thrombin-Antithrombin Complex Antibody:

$$V_T - V_A = V_D$$

Where:

C_S = Starting concentration (X) of stock Biotinylated Thrombin-Antithrombin Complex Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Thrombin-Antithrombin Complex Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Thrombin-Antithrombin Complex Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Thrombin-Antithrombin Complex Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Thrombin-Antithrombin Complex Antibody to prepare 1X Biotinylated Thrombin-Antithrombin Complex solution for assay procedures

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Thrombin-Antithrombin Complex Antibody stock

C_F = 1X Biotinylated Thrombin-Antithrombin Complex Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

(1X/50X) x 3,520 µL = 70.4 µL

3,520 µL - 70.4 µL = 3,449.6 µL

V_A = 70.4 µL total volume of (X) stock Biotinylated Thrombin-Antithrombin Complex Antibody required

V_D = 3,449.6 µL total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated Thrombin-Antithrombin Complex Antibody solution for assay procedures.

3. First spin the Biotinylated Thrombin-Antithrombin Complex Antibody vial to collect the contents at the bottom.
4. Add calculated amount VA of stock Biotinylated Thrombin-Antithrombin Complex Antibody to the calculated amount VD of 1X Assay Diluent N. Mix gently and thoroughly.

1X SP Conjugate: Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Δ Note Any remaining solution should be frozen at -20°C.

Standard Preparation

- Prepare serially diluted standards immediately prior to use.
- Always prepare a fresh set of standards for every use.

- Any remaining standard should be stored at -20°C after reconstitution and used within 5 days.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitution of the Thrombin-Antithrombin Complex Standard vial to generate a 120 ng/mL Thrombin-Antithrombin Complex Standard #1.
2. First consult the Thrombin-Antithrombin Complex Standard vial to determine the mass of protein in the vial.
3. Calculate the appropriate volume of 1X Diluent N to add when resuspending the Thrombin-Antithrombin Complex Standard vial to produce a 120 ng/mL Thrombin-Antithrombin Complex **Standard #1** by using the following equation:
   C_S = Starting mass of Thrombin-Antithrombin Complex Standard (see vial label) (ng)
   C_F = 120 ng/mL Thrombin-Antithrombin Complex **Standard #1** final required concentration
   V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 120 ng of Thrombin-Antithrombin Complex Standard in vial
C_F = 120 ng/mL Thrombin-Antithrombin Complex **Standard #1** final concentration
V_D = Required volume of 1X Diluent N for reconstitution
(110 ng / 120 ng/mL) x 1,000 = 1,000 µL

4. First briefly centrifuge the Thrombin-Antithrombin Complex Standard Vial to collect the contents on the bottom of the tube.
5. Reconstitute the Thrombin-Antithrombin Complex Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 120 ng/mL Thrombin-Antithrombin Complex Standard #1. Mix gently and thoroughly.
6. Allow the reconstituted 120 ng/mL Thrombin-Antithrombin Complex Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
7. Label six tubes #1 – 6.
8. Add 240 µL of 1X Diluent N to tube #2 – 6.
9. To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.
10. To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently.
11. Using the table below as a guide, prepare subsequent serial dilutions.
12. 1X Diluent N serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	Human Thrombin-Antithrombin Complex (ng/mL)
1	Step 1 of Standard Preparation		120
2	120 µL Standard #1	240	40
3	120 µL Standard #2	240	13.33
4	120 µL Standard #3	240	4.44
5	120 µL Standard #4	240	1.48
6 (Blank)	N/A	240	0

Sample Preparation

Please note that due to the chemistry of this kit, the provided standard cannot be used for spike recovery.

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an anticoagulant).

Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Diluent N; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1X. If necessary, dilute samples into Diluent N between 1:2 to 1:10. The user should determine optimal dilution factor depending on application needs. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

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 well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

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 instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Thrombin-Antithrombin Complex Standard or sample per well. Gently tap late to coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

4. Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash, six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µL of 1X Biotinylated Thrombin-Antithrombin Complex Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for one hour.
6. Wash microplate as described above.
7. Add 50 µL of 1X SP Conjugate to each well and incubate for 20 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 25 minutes or till the optimal blue color density develops
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
12. Analyze the data as described below.
13. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Sample Values

Sensitivity

The minimum detectable dose (MDD) of Thrombin-Antithrombin Complex is typically ~0.78 ng/ml.

Precision

	Intra-assay Precision	Inter-Assay Precision
CV (%)	5.4	10.4

Recovery

Note: It is not recommended to perform spike recovery in **plasma samples** due to large amount of endogenous Anti-thrombin III (mg/ml range) which will saturate the capture antibody and interfere with the binding of the TAT standard

Standard Added Value	1.5 - 40 ng/ml
Recovery (%)	90-114 %
Average Recovery (%)	96 %

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.
Plasma samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)	
Dilution Factor	**Plasma**
1:1	98
1:2	94
1:4	106

Species Reactivity

This kit recognizes Thrombin-Antithrombin Complex. 10% FBS in culture media will not affect the assay.

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	<40
Mouse	None
Rat	<15
Swine	<15
Rabbit	None

Typical Data

Data provided **for demonstration purposes only**. A new curve must be generated.

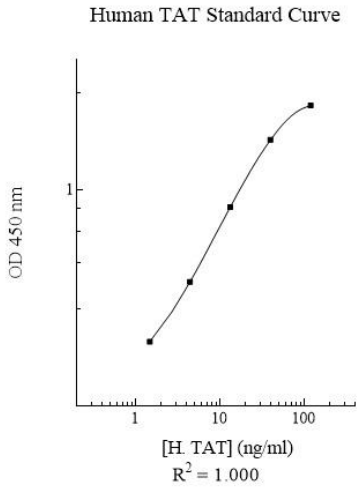

Figure 1. Example of Thrombin-Antithrombin Complex standard curve. The standard curve was prepared as described in the Standard Preparation section. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. plasma vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
Large CV	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. plasma vs. cell extract)	Detection may be reduced or absent in untested sample types

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)