



# SinoGeneClon Biotech Co.,Ltd

## INTRODUCTION

### Human Antithrombin III(AT III)ELISA Kit

FOR RESEARCH USE ONLY. Not for clinical diagnosis use

Catalog No : SG-13666

For the quantitative determination of Human AT III concentrations.

Reactivity:	Human
Method Type:	Sandwich ELISA Detection
Quantity:	96 tests
Sample type:	serum, plasma, Urine,tissue homogenates, cell culture supernates
Detection range :	3.75 ng/ml- 100 ng/ml
Sensitivity:	1 ng/ml

### Components:

Assay plate (12 × 8 coated Microwells)	1
Standard: 135 ng/ml	1×0.5ml
Standard Diluent	1×1.5ml
HRP-Conjugate Reagent	1×6ml
Sample Diluent	1×6ml
Chromogen Solution A	1×6ml
Chromogen Solution B	1×6ml
Stop Solution	1×6ml
Wash Solution	1×20ml×30 fold
User manual	1
Adhesive Strip	2

### Product Principle:

The kit is for the quantitative level of Human AT III in the sample, adopt purified AT III antibody to coat microtiter plate, make solid-phase antibody, then add AT III to wells, Combine AT III antibody with labeled HRP to form antibody-antigen -enzyme-antibody complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of AT III in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### Specimen requirements:

- 1.Serum-coagulation at room temperature for 10-20 min, centrifuge at the speed of 2000-3000 rpm for 20-min. Collect supernatant, if precipitation appeared, Centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- 2.Plasma-use suited EDTA or citrate plasma as an anticoagulant, centrifuge at the speed of 2000-3000 rpm for 20-min. Collect supernatant, if precipitation appeared, centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay .
- 3.Urine-collect sue a sterile container, centrifuge at the speed of 2000-3000 rpm for 20-min. Collect supernatant, if precipitation appeared, Centrifuge again. The Operation of Hydrothorax and cerebrospinal fluid reference to it. Assay immediately or aliquot and store samples at -20°C or -80°C.
- 4.Cell culture supernatant-detect secretory components, Remove particulates by centrifugation for 20-min at the speed of 2000-3000 rpm, collect supernatant . detect the composition of cells, dilute cell suspension with PBS (PH7.2-7.4), Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 rpm. collect supernatant, If precipitation appeared, Centrifugal again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.



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5. Tissue samples- After cutting samples, check the weight, Pipette PBS(PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting, Pipette PBS(PH7.4), homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 rpm. Collect supernatant. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

Note:

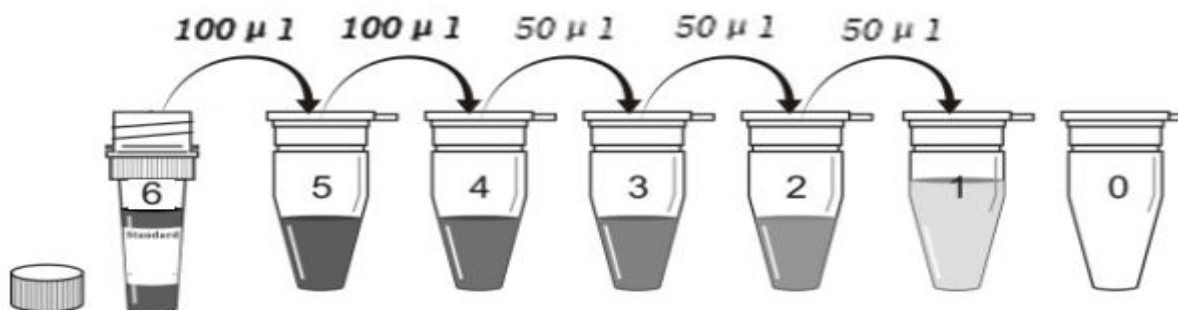
1. Extract as soon as possible after Samples collection, and should be tested as soon as possible after the extraction. If not, samples must be stored at -20°C ( $\leq 1$  month) or -80°C ( $\leq 2$  months) to avoid loss of bioactivity and contamination.
2. Can't detect the sample which contain NaN<sub>3</sub>, because NaN<sub>3</sub> inhibits HRP active.

## Reagent preparation

1: Wash Buffer (1x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (30 x) into deionized or distilled water to prepare 600 ml of Wash Buffer (1 x).

2: Standard

Dilute the standard: Pipette 50 $\mu$ l standard diluent in each tube. Pipette 100 $\mu$ l standard (135 ng/ml) in the fifth tube. And take out 100 $\mu$ l from the fifth tube into the fourth. Pipette 50 $\mu$ l from the fourth tube to the third tube and produce dilution series as below. The undiluted Standard serves as the high standard (135 ng/ml). Sample Diluent serves as the zero standard (0 ng/ml).



Tube	6	5	4	3	2	1	0
ng/ml	135	90	60	30	15	7.5	0

## Assay procedure:

- Step 1: Prepare all reagents, working standards, Blank and samples as directed in the previous sections.
- Step 2: Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C
- Step 3: Pipette standard 50 $\mu$ l to testing standard well, Pipette Sample diluent 40 $\mu$ l to testing sample well, then add testing sample 10 $\mu$ l (sample final dilution is 5-fold), Pipette sample to wells, don't touch the well wall as far as possible, and mix gently.
- Step 4: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37°C.
- Step 5: Configure liquid: Dilute wash solution 30-fold with distilled water.
- Step 6: Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times.
- Step 7: Add enzyme: Pipette HRP-Conjugate reagent 50 $\mu$ l to each well, except blank well.
- Step 8: Incubate: Operation with 4
- Step 9: Washing: Operation with 6.
- Step 10: Color: Pipette Chromogen Solution A 50 $\mu$ l and Chromogen Solution B 50 $\mu$ l to each well, avoid the light preservation for 15 min at 37°C.
- Step 11: Stop the reaction: Pipette Stop Solution 50 $\mu$ l to each well, stop the reaction (the blue change to yellow).
- Step 12: Calculate: take blank well as zero. Read absorbance at 450nm after pipette Stop Solution within 15min.



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### Calculation of result:

Take the standard concentration as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding concentration according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the result is the sample actual concentration.

Graphical Representation as following:



### Expiration:

Twelve months [see label on the outer box for the specific date]

### Storage conditions:

The unopened kit shall be stored at [2-8 °C]

For opened kit can be stored at [2-8 °C] for up to 1 month. If not be used recently, the standard should be kept in -20 °C

### Precision

**Intra-assay Precision (Precision within an assay):** 3 samples with low, middle and high level Human AT III were tested 20 times on one plate, respectively.

**Inter-assay Precision (Precision between assays):** 3 samples with low, middle and high level Human AT III were tested on 3 different plates, 8 replicates in each plate.

$CV(\%) = SD/\text{mean} \times 100$

**Intra-Assay:**  $CV < 8\%$

**Inter-Assay:**  $CV < 10\%$

### Attention:

- 1: The kit takes out from the refrigeration should be balanced 15-30 minutes in the room temperature, if the coated ELISA plates have not been used up after opening, the plate should be stored in sealed bag.
- 2: Washing buffer will Crystallization separation, it can be heated in water to dissolve.
- 3: Pipette sample with pipettors each step, and proofread its accuracy frequently to avoid the experimental error. Pipette sample within 5 min, if the number of sample is big, recommend using multichannel pipettor.
- 4: If the testing material concentration is excessively high (The sample OD is higher than the first standard well), please dilute the sample (n-fold).
- 5: Adhesive Strip only limits the disposable use to avoid cross-contamination.
- 6: The substrate should evade the light to be preserved.
- 7: Please refer to the user instruction strictly, the test result determination must take the microtiter plate reader as a standard.
- 8: The preparation of samples and all the reagents should refer to infective material process.
- 9: Do not mix reagents with those from other lots.



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### **Washing method:**

**Manually washing method:** shake away the remained liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

**Automatic washing method:** if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance .

### **If You Have Problems**

**Technical Service Contact information**

**Email:** [tech@sinogeneclon.com](mailto:tech@sinogeneclon.com)

**Web:** [www.sinogeneclon.com](http://www.sinogeneclon.com)

**In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).**