APC313Hu01 100μg
Active Antithrombin (AT)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Natural Extract.
Host: Human
Subcellular Location: extracellular space.
Purity: >94% as determined by SDS-PAGE.
Purification Methods: Salt co-precipitation and ionic-Exchange chromatography.
Traits: Freeze-dried powder
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted Molecular Mass: 62.0kDa
Accurate Molecular Mass: 62kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[ACTIVITY]

Antithrombin III, also known as Serpin C1, is a member of the Serpin superfamily of the serine protease inhibitors. It is the principal plasma Serpin of blood clotting proteases and inhibits thrombin as well as several factors such as Xa. Similar to Serpins A5 and D1, its thrombin inhibitory activity is enhanced by heparin. Hereditary and acquired Serpin C1 deficiency is the cause of an increased thrombotic tendency in many cases. For example, acquired Serpin C1 deficiency is a common condition in sepsis, after major trauma or surgery. The activity of recombinant human antithrombin III was measured by its ability to inhibit thrombin cleavage of a fluorogenic peptide substrate Boc-VPR-AMC in the assay buffer 50 mM Tris, 10 mM CaCl2, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5. Thrombin was diluted to 0.5 U with heparin at 50 μg/ml in the assay buffer and 10 ul different concentrations of recombinant human antithrombin III (MW: 50 KD) was incubated with 10 ul diluted thrombin at 37 ℃ for 30 minutes. Loading 50 μL of the incubated mixtures which were diluted five-fold in assay buffer into empty wells of a plate, and start the reaction by adding 50 μL of 200 μM substrate. Include a substrate blank containing 50 μL of assay buffer and 50 μL of 200 μM substrate. Then read at excitiation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes. The result was shown in Figure 1 and it was obvious that recombinant human antithrombin III significantly decreased thrombin activity. The inhibition IC50 was <8 nM.

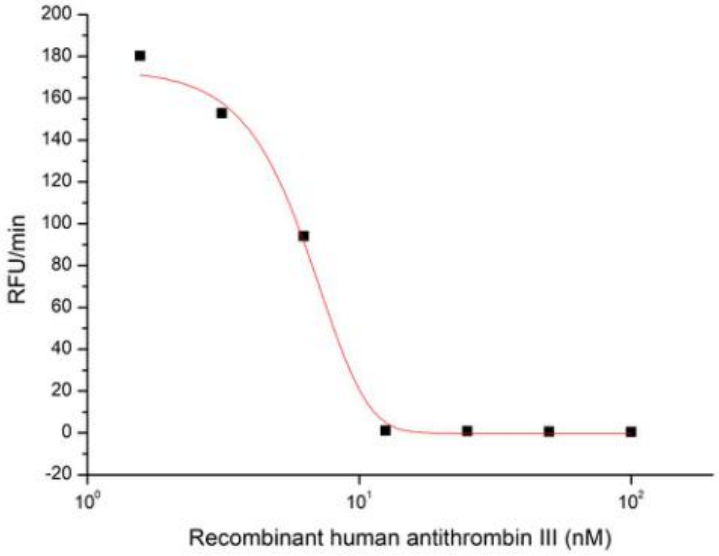

Figure 1. Inhibition of thrombin activity by recombinant human antithrombin III

[IDENTIFICATION]

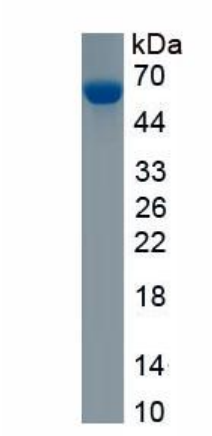

Figure 2. SDS-PAGE

Sample: Active recombinant AT, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.