# HUMAN ANTITHROMBIN NEUTRALIZATION SET

## REF 5D-19104

*Matched-Pair Antibody Set for Neutralization of human Antithrombin (ATIII) in Plasma*

**For Research Use Only. Not for Use in Diagnostic Procedures.**

10 Tests
Store at 2-8°C



### DESCRIPTION OF ANTITHROMBIN (ATIII)

Antithrombin, also known as Antithrombin III (ATIII), is a member of the SERPIN family of proteinase inhibitors and the primary inhibitor of thrombin in plasma. It is produced in the liver and circulates in plasma at ~200 µg/ml (~3.5 µM). Antithrombin inhibits a broad spectrum of serine proteases including thrombin, activated forms of factor X, factor IX, factor XI, factor XII, as well as kallikrein, plasmin and urokinase. Enzyme inhibition by antithrombin occurs through proteolytic cleavage at $Arg^{385}$-$Ser^{386}$ and subsequent rapid formation of a stable, inactive 1:1 enzyme-antithrombin complex. Heparin has a profound accelerating effect on the inhibitory activity of antithrombin towards some enzymes. The rate of inhibition of thrombin and of activated factor X is increased 1000-fold in the presence of optimal concentrations of heparin, whereas heparin has relatively little effect on the inhibition rate of activated factor XI, activated factor XII and kallikrein. Antithrombin is a single chain molecule with a molecular weight of 59 kDa. Interaction with thrombin results in an SDS-stable binary complex of 96 kDa[1-3].

### PRINCIPLE OF ATIII NEUTRALIZATION

Polyclonal sheep antibody to human ATIII is added directly to human plasma and allowed to incubate for 20 minutes. During this time the antibody will bind to and inactivate (neutralize) the ability of ATIII to inhibit thrombin or activated factor X, even in the presence of heparin. Clot-based tests can then be performed on the neutralized sample. To control for the addition of antibody volume and buffer, a control antibody (non-immune sheep IgG, at the same concentration and in the same buffer) is added separately to a duplicate sample and the test is repeated.

### PRODUCT DESCRIPTION

1. Neutralizing IgG (5D-19104-T): One purple-capped vial containing 0.1 ml of sheep antibody (IgG) to human ATIII.
2. Control IgG (5D-19104-C): One white-capped vial containing 0.1 ml of sheep IgG prepared from non-immune serum for use as a negative control.

Buffer: Both the neutralizing and control IgG have the same protein concentration in 0.02 M HEPES, pH 7.4, 0.01 M $Na_3$-Citrate, 0.15 M NaCl and 0.02% (w/v) $NaN_3$.

**Storage:** Refrigerate at 2-8°C

### TEST PROCEDURE

1. Prepare duplicate test tubes, each containing 90 µL of plasma.
2. To the tube labelled test, add 10 µL of test IgG (5D-19104-T).
3. To the tube labelled control, add 10 µL of control IgG (5D-19104-C)
4. Mix contents of both tubes and incubate at room temperature for 20 minutes. Both plasmas may be used directly or placed on ice for up to 4 hours.

In the quality control of this product, Thrombin Clot Times were performed as follows:

- 5 µL of 3 U/mL standard heparin (final = 0.3 U/mL in plasma)
- 50 µL of normal citrated plasma
- 150 µL of Seegers solution (28 mM Imidazole, 1.7 % (w/v) acacia, 3.7 mM CaCl2, 57 mM NaCl, pH 7.3)
- 50 µL of human thrombin, adjusted to produce a clot time of approximately 30 seconds in the absence of heparin

Clot times were measured using the ST-4 BIO analyzer (Diagnostica Stago) according to manufacturer's directions.

### TECHNICAL NOTES

Under the conditions described above, greater than 99% of ATIII in normal plasma is neutralized. This is confirmed using ATIII deficient plasma (product 5D-42104F).
The above conditions are only intended for use in screening assays of compounds that may exert anticoagulant activity through ATIII. The use of these reagents for quantitative determination of ATIII activity is not recommended.

### REFERENCES

1. Damus PS, Rosenberg RD; Antithrombin – Heparin Cofactor; Methods in Enzymology 45, pp 653-669, 1976.
2. Harpel PC; Blood Proteolytic Enzyme Inhibitors: Their Role in Modulating Blood Coagulation and Fibrinolytic Enzyme Pathways; in Hemostasis and Thrombosis, eds. RW Colman, J Hirsh, VJ Marder and EW Salzman, pp. 738- 747, J.B. Lippincott Co., Philadelphia PA, USA, 1982.
3. Griffith MJ, Noyes CM, Church FC; Reactive Site Peptide Structural Similarity between Heparin Cofactor II and Antithrombin III; JBC:260, pp 2218-2225, 1985.



5-Diagnostics AG
Heuberg 7, 4051 Basel,
Switzerland
www.5-diagnostics.com
info@5-diagnostics.com