



## Antithrombin (human) Neutralization Matched Pair Antibodies (10 Tests)

Ref#: AT-NU10  
Lot#: xxxxxx  
Exp Date: xxxx-xx

Store at +2 to +8°C

For Research Use Only  
Not for Use in Diagnostic Procedures  
For *in vitro* use only

### **INTENDED USE:**

Matched Pair Antibody Set is intended for neutralization of Antithrombin (ATIII) in plasma or therapeutic concentrates. **The results obtained should be for research purposes only and not used for patient diagnosis or treatment.**

### **SUMMARY:**

Human Antithrombin, also known as Antithrombin III (ATIII), is a proteinase inhibitor synthesized in the liver and composed of a single polypeptide chain. 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. Antithrombin concentration in human plasma is about 200 µg/mL (3.5 µM).

### **PRINCIPLE OF NEUTRALIZATION:**

Polyclonal sheep antibody to human ATIII is added directly to human plasma or therapeutic concentrate and incubated for 20 minutes. During this time the antibody will bind 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.

### **REAGENTS:**

#### **Required Materials provided (enough for 10 Tests):**

**A: Neutralizing IgG (AT-NU10-A).** 1 vial of 0.1 mL sheep IgG antibody specific for ATIII. Buffered in 20 mM HEPES / 0.15 M NaCl / 0.01 M sodium citrate / pH 7.2. Purple cap.

**NC: Neutralizing Control (AT-NU10-NC).** 1 vial of 0.1 mL sheep IgG antibody prepared from non-immune serum. Buffered in 20 mM HEPES / 0.15 M NaCl / 0.01 M sodium citrate / pH 7.2. For use as a negative control. White cap.

*Note: Antibodies should be stored at +2 to +8°C. Vials should be tightly capped.*

### **PROCEDURE**

1. Prepare test-tubes in duplicate (1x "Test" and 1x "Control"), each with 90 µL of plasma sample.
2. Add 10 µL of [A] Neutralizing IgG to the "Test" tube
3. Add 10 µL of [NC] Neutralizing Control to the "Control" tube
4. Mix and incubate @ ambient temperature for 20 minutes.

Neutralized plasma can be used directly or placed on ice for up to 4 hours.

### **RESULTS:**

Under the conditions described above, greater than 99% of ATIII in normal plasma is neutralized. This is confirmed using ATIII deficient plasma. The above conditions are only intended for use in screening assays of compounds that may exert anticoagulant activity through ATIII.

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