



Chrom ATIII

For the quantitative determination of antithrombin III by chromogenic method

SUMMARY

Antithrombin III (ATIII) is a physiological inhibitor of the coagulation system belonging to the serpin family, inhibitors of serine proteases. Although it is the major inhibitor of thrombin (FIIa) it also inhibits activated Factor X (FXa), activated Factor IX (FIXa), activated Factor XI (FXIa), activated Factor XII (FXIIa), kallikrein, plasmin and the complex Factor VIIa/tissue factor. Heparin accelerates the inactivation of IIa and Xa by ATIII, action known as heparin cofactor activity.

ATIII deficiency can be acquired or inherited and is associated with increased risk of deep venous thrombosis, pulmonary embolism and arterial thrombosis.

As a reactant acute phase protein, increased levels of ATIII might be observed during inflammatory processes, infections and in the presence of tumors.

PRINCIPLE

Antithrombin III in the sample inhibits excess thrombin added in presence of heparin during the first reaction phase. Residual thrombin measured in the second phase of the reaction through its amidolytic activity on a specific chromogenic substrate at 405 nm, is inversely proportional to the concentration of ATIII in the sample.

PROVIDED REAGENTS

- A. Reagent A:** vial containing ~43 IU thrombin/vial. Lyophilized.
- B. Reagent B:** chromogenic substrate thrombin-specific containing 10 μ mol substrate/vial. Lyophilized.
- C. Reagent C:** 0.9% ClNa solution

NON-PROVIDED REAGENTS

- Distilled water.
- Wiener lab.'s Coagulation Control N and Coagulation Control P.
- Wiener lab.'s Coagulation Calibrator.

INSTRUCTIONS FOR USE

Reagent A: reconstitute with 20 ml distilled water. Let stand, not less than 10 minutes (preferably 30 minutes) at room temperature before use.

Reagent B: reconstitute with 8 ml distilled water. Let stand, not less than 10 minutes (preferably 30 minutes) at room temperature before use.

Reagent C: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.
Use the reagents according to the working procedures for

clinical laboratories.

All reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents are stable in refrigerator (2-10°C) until the expiration date shown on the box.

After reconstitution they are stable:

Reagent	37°C	RT (<25°C)	2-10°C	-20°C
Reagent A	1 day	4 weeks	4 months	6 months
Reagent B	1 day	4 weeks	6 months	1 year

SAMPLE

Citrated plasma

a) Collection: obtain blood carefully (avoiding stasis or trauma) and place into a tube with anticoagulant in an exact 9+1 proportion (e.g.: 4.5 ml blood + 0.5 ml anticoagulant). Mix gently. Centrifuge for 15 minutes at 2,500 g and separate plasma before 30 minutes.

b) Additives: use Wiener lab's Anticoagulante TP or 130 mmol/l (3.8%) or 109 mmol/l (3.2%) sodium citrate to obtain plasma.

c) Known Interfering Substances: do not use EDTA or heparin to obtain plasma.

Refer to Young, D.S. in references for effect of drugs on the present method.

d) Stability and Storage Instructions: plasma must be stored at room temperature until the test is performed. This period should not last for more than 3 hours. In case the sample cannot be processed within this period, the plasma can be frozen up to 1 month at -20°C.

REQUIRED MATERIAL (non-provided)

- Hemolysis tubes
- Pipettes and micropipettes for measuring the stated volumes
- Water bath at 37°C
- Stopwatch
- Spectrophotometer or coagulation analyzer.

ASSAY CONDITIONS

- Wavelength: 405 nm
- Reaction temperature: 37°C
- Reaction time: 270 seconds
- Final Volume: 800 μ l (endpoint technique) or 600 μ l (kinetic technique).

PROCEDURE

Predilute plasma samples in the 1:80 ratio with Reagent C (25 μ l sample + 2.0 ml Reagent C). Store at room temperature until the test is performed.
Preheat reconstituted Reagents A and B at 37°C.

Kinetic technique

In a tube labeled U (Unknown) or C (Calibrator) place:

	U or C
Prediluted sample	200 μ l
Reagent A	200 μ l

Mix and incubate for exactly 90 seconds at 37°C. Then add:

Reagent B	200 μ L
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Mix and incubate for exactly 180 seconds at 37°C. Then add:

Mix and read absorbance at 405 nm during 3 minutes ($\Delta A/\text{min}$) recording absorbances every minute. Calculate antithrombin III activity (%) using the absorbance change per minute and interpolating the calibration curve.

CALIBRATION CURVE

Predilute the Coagulation Calibrator in a 1:80 ratio with Reagent C in the same way that the samples. From the prediluted Coagulation Calibrator prepare the following serial dilutions: 1/1, 1/2, 1/4 also with Reagent C and using the latter as a point value of 0%. Plot the change in absorbance obtained from each dilution according to % activity of antithrombin III. Antithrombin III concentration is determined by interpolation on the calibration curve.

REFERENCE VALUES

80-120% (0.80-1.20 IU ATIII/ml)

Each laboratory should establish its own reference values based on the techniques and devices used.

QUALITY CONTROL METHOD

Wiener lab.'s Coagulation Control N and Coagulation Control P.

PROCEDURE LIMITATIONS

See Known Interfering Substances and Stability and Storage Instructions under SAMPLE.

Recalibration is required for each batch of reagents and for each instrument used.

PERFORMANCE

a) Reproducibility: reproducibility was determined with various samples (in series and day-to-day). The following results were obtained:

Intra-assay precision

Level	S.D.	C.V.
105.5%	2.21%	2.12%
54.7%	1.61%	2.95%

Inter-assay precision

Level	S.D.	C.V.
103.3%	1.75%	1.69%
53.9%	1.275%	2.37%

b) Measuring range: 0 - 130%

c) Sensitivity: detection limit is 4.0%

PARAMETERS FOR AUTOANALYZERS

Refer to the specific applications of each autoanalyzer.

WIENER LAB PROVIDES

1 x \rightarrow 20 ml Reagent A

1 x \rightarrow 8 ml Reagent B

2 x 25 ml Reagent C

(Cat. N° 1705012)

REFERENCES

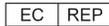
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- Tolefsen, D. M. and Blank, M. K. Journal of Clinical Investigations 1981. 68: 589-596.
- Friberger, P., Egberg, N., Holmer, E., Hellgren, M. and Blomback, M. Thrombosis Research 1982. 25: 433-436.
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Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Authorized representative in the European Community



"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution



Contents



Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use



Calibrator



Control



Positive Control



Negative Control



Catalog number

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