

ACTICHROME® AT III

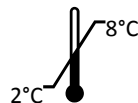
REF 838

*chromogenic assay for measuring
anti-thrombin III activity in human plasma*

IVD



Σ 60



INTENDED USE

ACTICHROME® AT III is intended for the quantitative determination of antithrombin III in human plasma by chromogenic assay. The assay is intended for in vitro diagnostic use.

EXPLANATION OF THE TEST

Antithrombin III is an inhibitor of plasma serine proteases. An important function of antithrombin III is the inhibition of thrombin activity. Normally the rate of thrombin inhibition by antithrombin III is slow (progressive antithrombin activity). However, the rate of inhibition can be enhanced several thousand-fold in the presence of heparin (heparin cofactor activity).

Tolfesen and Blank have reported another rapid heparin-dependent thrombin inhibitor, Heparin Cofactor II, in human plasma. This protein can interfere with antithrombin III determinations especially at high (2 USP units/mL) heparin concentrations. In order to confer specificity to antithrombin III the present assay system uses a lower (1.0 USP units/mL) final heparin concentration where heparin-enhanced inactivation of thrombin by heparin cofactor II is negligible. In addition, human heparin cofactor II reacts more readily with human thrombin than with bovine thrombin (Friberger et al.). Thus, further specificity for antithrombin III is imparted in the present assay system by the use of bovine thrombin.

PRINCIPLE OF THE METHOD

In the present two-stage method (Odegard, et al.), thrombin is added to a plasma dilution containing antithrombin III in the presence of excess heparin. After an initial incubation (stage 1) residual thrombin is determined with a thrombin-specific chromogenic substrate (stage 2). The residual thrombin activity is inversely proportional to the antithrombin III concentration of the plasma.

REAGENTS

The kit contains sufficient reagents to perform 60 tests using semi-micro methodology.

R1 Bovine Thrombin: 6 vials (lyophilized).

R2 SPECTROZYME® TH: 6 vials each containing 1.8 µmoles thrombin substrate (lyophilized).

R3 Assay Buffer: 6 vials each containing 5 mL of buffer, 10-fold concentrate. Working strength buffer contains 50 mM Tris-HCl, 175 mM NaCl, 7.5 mM Na⁺EDTA and 1.0 USP units/mL sodium heparin, pH 8.4.

WARNINGS AND PRECAUTIONS

Do not use kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the kit components. Do not mouth pipette or ingest reagents.

Bovine Thrombin	Warning		H315, H319; P264, P280, P302 + P352, P305 + P351 + P338, P337 + P313		
SPECTROZYME® TH	Warning		<table><tr><td>CONT</td><td>H-D-cyclohexylalanyl-alanyl-arginine-para-nitroanilide diacetate salt</td></tr></table> H315, H319, H335, P261, P264, P280, P302 + P352, P305 + P351 + P338, P337 + P313	CONT	H-D-cyclohexylalanyl-alanyl-arginine-para-nitroanilide diacetate salt
CONT	H-D-cyclohexylalanyl-alanyl-arginine-para-nitroanilide diacetate salt				
Assay Buffer	-	-	Observe good laboratory hygiene practices.		

Hazard Statements:
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

Precautionary Statements:
P261 Avoid breathing dust.
P264 Wash thoroughly after handling.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P305 + P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT PREPARATION AND STORAGE

Intact vials of reagents are stable until the label expiry date when stored at 2° - 8°C.

R1 Bovine Thrombin: Reconstitute with 2 mL of filtered deionized water. Reconstituted reagent is stable for 1 week at 2-8°C and for up to one month at -20°C

R2 SPECTROZYME TH: Reconstitute with 2 mL of purified water filtered deionized water. Reconstituted reagent is stable for 1 week at room temperature, 2 months at 2° - 8°C and 6 months at -20°C (aliquot and freeze).

R3 Assay Buffer: Dilute to 50 mL with filtered deionized water purified water. Working strength buffer is stable for 1 week at room temperature and for 1 month at 2° - 8°C.

SPECIMEN COLLECTION AND PREPARATION

Citrate collected platelet poor plasma may be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays-Approved Guideline", CLSI Document H21-A5, Vol. 28, No. 5, January 2008. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 1,500 x g for 15 minutes.
3. Plasma should be stored at 2° - 8°C and assayed within 2 hours. Alternatively, plasma may be stored at -20°C for up to 1 month.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2° - 8°C and assayed within 24 hours.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

filtered deionized H₂O
0-200 µL, 200-1000 µL single pipettes
Plastic test tubes
Laboratory timer
37°C wet or dry bath
50% glacial acetic acid
Spectrophotometer operable at 405 nm
Pooled normal plasma or commercial reference plasma

Assay Calibration

Pooled normal human plasma (at least 10 normal donors), which has been collected in the same way as plasmas to be tested, may be used for preparation of the antithrombin III standards. Since oral contraceptives and other estrogen/progesterone preparations may affect antithrombin III levels, plasma from users of such preparations should be excluded from the pool. Commercially prepared plasma standard in which antithrombin III has been determined may also be used.

Prepare plasma antithrombin III standards and unknown plasma samples as follows:

Standard	Volume of Pooled Normal Plasma	Volume of Assay Buffer
100%	25 µL	1000 µL
50%	500 µL of 100% Standard	500 µL
0%	0 µL	1000 µL
Unknowns	25 µL Plasma Specimen	1000 µL

Assay Procedure – Endpoint Method

Endpoint Method

1. Add 200 µL of standard or unknown plasma to a plastic tube.
2. Incubate at 37°C for 2-4 minutes.
3. Add 200 µL of Bovine Thrombin.
4. Mix and incubate at 37°C for 1 minute.
5. Add 200 µL of SPECTROZYME TH.
6. Mix and incubate at 37°C for 1 minute.
7. Add 200 µL of 50% glacial acetic acid.
8. Mix.
9. Add 200 µL of water* (optional).

Read the absorbance at 405 nm in a 1 cm semi-microcuvette against a blank prepared in the following order:

- 200 µL acetic acid
- 200 µL standard dilution
- 200 µL Bovine Thrombin
- 200 µL SPECTROZYME TH
- 200 µL water* (optional)

(*Some spectrophotometers require a minimum of 1 mL volume in the cuvette.)

Multiple Simultaneous Determinations - As many as ten determinations can be performed simultaneously with the same stopwatch by staggering pipetting steps at five second intervals.

Assay Procedure - Kinetic Method

A kinetic analyzer may be used to measure the initial rate of hydrolysis of the chromogenic substrate. The procedure to be used is as follows:

1. Add 5 µL of standard or unknown plasma to 200 µL of Dilution Buffer.
2. Incubate at 37°C for 2-4 minutes.
3. Add 200 µL of Bovine Thrombin.
4. Mix and incubate at 37 °C for 1 minute.
5. Add 200 µL of SPECTROZYME TH.
6. Measure rate of change of absorbance at 405 nm.

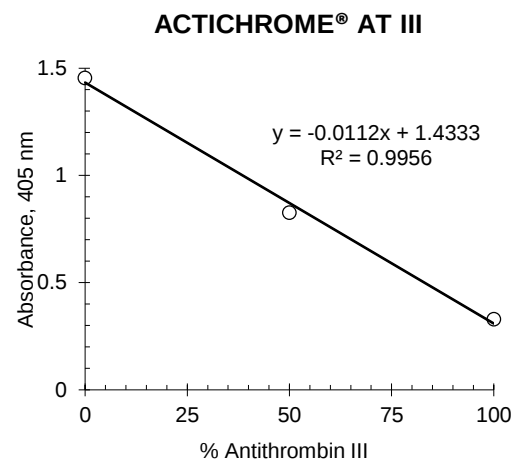
RESULTS

Representative Standard Curve

Plot the absorbance obtained for each antithrombin III standard against the percent of antithrombin III on linear graph paper. Interpolate the antithrombin III level of the unknown plasma sample from the calibration curve. If a commercial antithrombin III reference standard was used, adjust the antithrombin III value determined for the unknown plasma sample as follows:

$$\% \text{ AT III (adjusted)} = \% \text{ AT III (unknown plasma)} \times \% \text{ AT III (reference)} / 100$$

The calibration curve shown below is for example only. A new calibration curve must be constructed each time the assay is performed.



QUALITY CONTROL

Commercial antithrombin III reference plasma may be used for quality control of the assay. If commercial antithrombin III control plasma has been used to construct the calibration curve for the assay, then a different lot of control plasma should be used for quality control.

LIMITATIONS OF THE PROCEDURE

Icteric, lipemic and hemolyzed samples may interfere with the assay. If the unknown plasma is very icteric, a second blank containing the unknown plasma dilution instead of the standard dilution should be prepared and its absorbance subtracted from the absorbance obtained for the unknown antithrombin III determination

EXPECTED VALUES

The normal range of ATIII in plasma is 75%-125%. Activity levels of 30-60% may be observed in patients with hereditary ATIII deficiency. Several clinical conditions associated with acquired ATIII deficiency include liver disease, DIC, nephrotic syndrome, pulmonary embolism, stroke and thrombophlebitis. In addition, oral contraceptive use may reduce ATIII levels.

PERFORMANCE CHARACTERISTICS

Accuracy

In clinical studies comparing ACTICHROME ATIII to several other commercially available chromogenic antithrombin III kits the following correlation was observed:

$$\% \text{ ATIII (other assays)} = 0.93 \% \text{ ATIII (ACTICHROME)} + 5.9 \text{ (n=53, r = 0.80)}$$

Precision

The following estimates of precision (coefficient of variation) were observed using the semi-micro stopped end-point mode. Precision can be significantly improved using a kinetic mode.

% AT III	Coefficient of Variation	
	Intra-Assay (n=20)	Inter-Assay (n = 10)
100	4.4%	5.8%
50	3.4%	6.4%

Sensitivity

ACTICHROME AT III is sensitive to 10% antithrombin III.

Specificity

The specificity of the assay system has been established in studies employing plasma that has been selectively depleted of antithrombin III followed by addition of purified antithrombin III to achieve various antithrombin III concentrations.

TRACEABILITY OF CALIBRATORS AND CONTROL MATERIAL

Information on traceability of calibrators and control material is available upon request.

REFERENCES

1. Odegard, O. R., Lie, M. and Ablidgaard, U. *Thrombosis Research* 1975, **6**: 287-294.
2. Tolefsen, D. M. and Blank, M. K. *Journal of Clinical Investigations* 1981, **68**: 589-596.
3. Friberger, P., Egberg, N., Holmer, E., Hellgren, M. and Blomback, M. *Thrombosis Research* 1982, **25**: 433-436.

DEFINITION OF SYMBOLS

	Consult instructions for use		Batch code / Lot number
	Refer to SDS		Use By / Expiration Date
	In vitro diagnostic medical device		Catalog number
	Manufactured by		Contains sufficient for <n> tests
	Temperature limitation: Store at 2°C to 8°C		Contains...