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ACTICHROME® AT III

REF 838

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

IVD CE Σ 60 8°C 2°C

EC REP **Obelis s.a**
Boulevard Général Wahis 53, 1030 Brussels, BELGIUM

INTENDED USE

ACTICHROME® AT III is intended for the quantitative determination of antithrombin III activity 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).

Tolefsen 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

REF 838 contains sufficient reagents to perform 60 tests using a manual method.

R1 Bovine Thrombin: 6 vials (lyophilized).

R2 SPECTROZYME® TH: 6 vials (lyophilized).

R3 Assay Buffer: 6 vials , 5 mL, 10X concentrate.

WARNINGS AND PRECAUTIONS

This product contains animal source material. As no known test method can provide complete assurance that products derived from animal specimens will not transmit blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious specimen.

Bovine Thrombin	Warning		H315, H319; P264, P280, P302 + P352, P305 + P351 + P338, P337 + P313
SPECTROZYME® TH	Warning		CONT: H-D-cyclohexylalanyl-alanyl-arginine-para-nitroanilide diacetate salt H315, H319, H335, P261, P264, P280, P302 + P352, P305 + P351 + P338, P337 + P313
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°-8ºC.

R1 Bovine Thrombin: Reconstitute with 2 mL of filtered deionized water. Reconstituted bovine thrombin is stable for:

	1 week	4 weeks
	2°-8°C	-20°C

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R2 **SPECTROZYME TH:** Reconstitute with 2 mL of purified water filtered deionized water. Reconstituted substrate is stable for:

	1 week	4 weeks
	2°-8°C	-20°C

R3 **Assay Buffer:** Dilute the Assay Buffer to 50 mL with filtered deionized water. Working strength Assay Buffer is stable for:

	1 week	4 weeks
	2°-8°C	-20°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°-8ºC 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°-8ºC and assayed within 24 hours.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

Type 1 deionised water or distilled water
Special Coagulation Calibrator, REF C.BMD.SCC030-01ML-A
Special Coagulation Control Normal, REF C.BMD.SCCN180-01ML-A
Special Coagulation Control Abnormal, REF C.BMD.SCCA180-01ML-A
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, coagulation analyser

Assay Calibration

The Special Coagulation Calibrator, REF C.BMD.SCC030-01ML-A, or a 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.

Prepare Antithrombin III standards, controls and patient plasma samples as follows. Assay the standards, controls and samples immediately after preparation.

Standard*	Volume of Special Coagulation Calibrator	Volume of Assay Buffer
100%	25 µL	1000 µL
50%	500 µL of 100% Standard	500 µL
0%	0 µL	1000 µL
Patient Sample/Control	**Volume of Patient Sample/Control**	**Volume of Assay Buffer**
	25 µL	1000µL

*The actual value of the Antithrombin III Standards will depend on the lot specific value of the Special Coagulation Calibrator.

Assay Procedure

ACTICHROME AT III may be performed manually, or by using semi-automated or automated coagulation analysers.

BioMedica Diagnostics offers Instrument Applications for performing ACTICHROME AT III on several coagulation analysers. These Instrument Applications may contain platform specific programming and performance data which differ from that provided in this Instructions for Use. In these cases, the information contained in the Instrument Application supersedes the information in this Instruction for Use. Please consult the specific manufacturer's instrument manual for complete operating instructions.

Assay Procedure – Manual Method

Endpoint Method

1. Add 200 µL of standard or unknown plasma to a plastic tube.
2. Incubate at 37ºC for 2-4 minutes.
3. Add 200 µL of Bovine Thrombin.
4. Mix and incubate at 37ºC for 1 minute.
5. Add 200 µL of SPECTROZYME TH.
6. Mix and incubate at 37ºC 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.)

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Kinetic Method

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

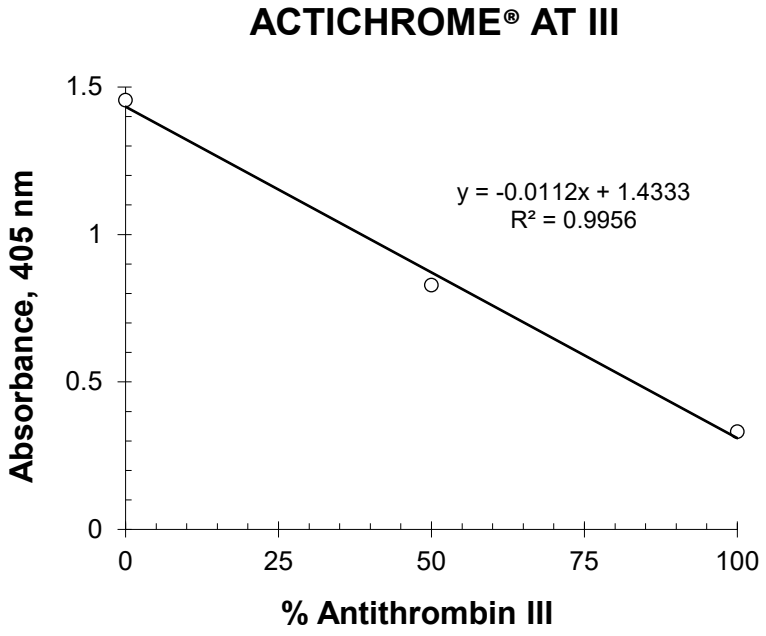
1. Add 5 µL of standard or unknown plasma to 200 µL of Assay Buffer.
2. Incubate at 37ºC for 2-4 minutes.
3. Add 200 µL of Bovine Thrombin.
4. Mix and incubate at 37ºC for 1 minute.
5. Add 200 µL of SPECTROZYME TH.
6. Measure rate of change of absorbance at 405 nm.

RESULTS

Representative Standard Curve

A standard curve is constructed by plotting the mean absorbance for each Antithrombin III standard versus its corresponding activity in percent. A standard curve should be generated each time the assay is performed. Draw the line of best fit between the points, typically a linear equation, for data analysis.

The following standard curve is for demonstration purposes only.



CALCULATION OF RESULTS

Interpolate the antithrombin III activity of the patient sample directly from the standard curve.

QUALITY CONTROL

Control plasmas should be included in the run whenever freshly reconstituted reagents are used. The Special Coagulation Control Normal, REF C.BMD.SCCN180-01ML-A, and the Special Coagulation Control Abnormal, REF C.BMD.SCCA180-01ML-A, may be used. The levels obtained for these control plasmas should fall within the specified ranges. If these controls plasmas fail to yield Antithrombin III levels within the specified range, the run should be repeated. Contact BioMedica Diagnostics if repeated assaying of these control plasmas continues to fail to yield Antithrombin III levels within the specified range.

LIMITATIONS OF THE PROCEDURE

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

EXPECTED VALUES

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

PERFORMANCE CHARACTERISTICS

Accuracy

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

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

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.

DEFINITIONS OF SYMBOLS

	Consult instructions for use		Warning
IVD	In vitro diagnostic medical device	2°C 8°C	Temperature limitation Store at 2°C to 8°C
LOT	Lot Number	REF	Catalog Number
	Expiration Date		Manufacturer
Σ n	Contains sufficient for <n> tests	CONT	Contains...
CE	CE mark	EC REP	European Union Authorized Representative