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TECHNOCHROM® ATIII

For research use only

GB

Complete reagent kit:

REF	5340224	TECHNOCHROM® ATIII Kit	~ 100 T.
REF	5340225	TECHNOCHROM® ATIII manual method Kit	~ 39 T.

Modular reagents:

REF	5340212	TECHNOCHROM® ATIII modular Reagent A1	2 x 6.5 IU
REF	5340214	TECHNOCHROM® ATIII modular Reagent A1	20 x 6.5 IU
REF	5340217	TECHNOCHROM® ATIII modular Reagent A2	2 x 43 IU
REF	5340219	TECHNOCHROM® ATIII modular Reagent A2	20 x 43 IU
REF	5340011	TECHNOCHROM® ATIII modular Substrate Th-1	2 x 10 µmol
REF	5340016	TECHNOCHROM® ATIII modular Substrate Th-1	20 x 10 µmol
REF	5340221	TECHNOCHROM® ATIII modular Buffer A1	100 mL

Symbols key			
	Manufacturer	AQUA	Distilled water
	Expiry date	DIL	Dilute or disolve
	Storage temperature	LOT	Lot
	Consult instructions for use	REF	Catalogue number
RUO	For research use only	SUB	Substrate
	Determinations		

Technoclone Herstellung von
Diagnostika und Arzneimitteln GmbH
Brunner Str. 67 - 1230 Vienna, Austria

TECHNOCHROM® ATIII

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PRODUCT DESCRIPTION

INTENDEND USE

The TECHNOCHROM® AT III Kit is a complete reagent kit suitable for the chromogenic determination of ATIII on auto-analyzers.
The TECHNOCHROM® AT III modular reagent is a system of reagents for the chromogenic determination optimized for the manual method (Reagent A1, Th-1 and Buffer A1) and different types of auto analyzers (Reagent A2 and Reagent Th-1).

PRINCIPLE

$ATIII_{sample}$ + Thrombin (Reagent A1 or A2) —Heparin→ [AT III . Heparin . Thrombin] complex + residual Thrombin

Substrate Th-1 —residual Thrombin→ release of p-Nitroanilin (405 nm)

The optimised combination of the Substrate Th-1 with Reagents A1 or A2 respectively is mentioned in the application sheet for the auto analyser used, which is available on request.

COMPOSITION

The composition of the modular reagents is:

TECHNOCHROM® AT III Reagent A1:	~ 6.5 IU Thrombin / vial
TECHNOCHROM® AT III Reagent A2:	~ 43 IU Thrombin / vial
TECHNOCHROM® AT III Substrate Th-1:	10 µmol / vial of the chromogenic SUB Th-1
TECHNOCHROM® AT III Buffer A 1:	100 ml Tris- Na_2EDTA- (3,03g/L) NaCl- (13g/L) Albumin- (2g/L) solution with pH 7,5.

The REF 5340224 TECHNOCHROM® ATIII **auto analyzer** Kit for ~ 100 T. contains:

vial(s)	reagents
1 x ~ 43 IU	TECHNOCHROM® AT III Reagent A2
1 x 10 µmol	TECHNOCHROM® AT III Reagent Th-1
2 x 25 mL	Sodium Chlorid Solution 0.9 %

The REF 5340225 TECHNOCHROM® ATIII **manual method** Kit for ~ 39 T. contains:

vial(s)	reagents
1 x ~ 6.5 IU	TECHNOCHROM® AT III Reagent A1
1 x 10 µmol	TECHNOCHROM® AT III Reagent Th-1
1 x 100 mL	TECHNOCHROM® AT III Buffer A 1

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water
- Buffer required for **manual method** and in combination with modular reagents

REF 5340221 TECHNOCHROM® ATIII modular Buffer A1 100 mL

- Buffer required for **auto analyzers** and in combination with modular reagents

REF 4847127 Sodium Chlorid Solution 0.9% 25 mL

- Control and Calibration Plasma **

REF 5020040 Coagulation Control N 5 x 1 mL

REF 5021055 Coagulation Control A 5 x 1 mL

REF 5220110 Coagulation Reference 5 x 1 mL

** or any other package sizes, special Ceveron® alpha or TECHNOCLOT® Control and Calibration reagents of Technoclone.

WARNING AND PRECAUTIONS

- For research use only
- All blood and plasma samples and products have to be regarded as potentially infectious and handled with appropriate care and in compliance with the biosafety regulations in force and must be disposed of in the same way as hospital waste.

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at + 2…8 °C.

Stability after reconstitution:

Reagent	+37 °C	RT*	+15 °C (Ceveron®)	+4 °C
Reagent A1	1 day	4 weeks	2 months	4 months
Reagent A2	1 day	4 weeks	2 months	4 months
Substrate Th-1	1 day	4 weeks	3 months	6 months

*=room temperature

Upon storage, caps should be screwed tightly.

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Mix 9 parts of venous blood and 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a RCF of at least 2500 (corresponding to DIN 58905). The plasma sample may not be stored at room temperature for more than three hours; otherwise the sample has to be frozen immediately after centrifugation. Stability at – 20°C: 1 month.

PREPARATION OF SAMPLES

Dilute sample in accordance to the application sheet for the auto analyzer.

PREPARATION OF REAGENT

All reagents including distilled water should have reached room temperature before use. In accordance to the application sheet for the auto analyzer, the lyophilized reagents are dissolved in distilled water or AT III buffer A1 and are ready for use after 10 minutes. (For standardization test: reconstitution time = 30 min).The Coagulation Reference has to be dissolved in distilled water in accordance to the enclosed table [AT III, method b) chromogenic substrate] and contains 1 IU AT III/ml (100% of normal).

PERFORMANCE OF THE TEST

Application sheets for auto analysers are available on request.

CEVERON

Technoclone provides application sheets for Ceveron® alpha. The application sheets contain analyser/assay specific handling and performance information which may differ from that provided in this instruction for use. In this case the information contained in the application sheet supersedes the information in this instruction for use. Please consult the instruction manual of the Ceveron® alpha.

MANUAL

Example: Manual Method using a Photometer
Wave length: 405 mn

Dissolve the AT III A1 in 21 mL AT III Buffer A1 and the AT III Substrate Th-1 in 8 mL distilled water. Preheat both reconstituted reagents to 37°C. Dilute plasma samples 1:81 with AT III Buffer A1 (0.025 mL sample + 2.0 mL buffer). Keep the plasma samples at room temperature. Measurements are done at 37°C.
Pipetting scheme: Pipette into plastic tubes or cuvettes:

Kinetic determination		Endpoint determination
50 µL 500 µL	Sample (1:81) AT III Reagent A1	50 µL 500 µL
90 sec. 200 µL	Incubation AT III Substrate Th-1	90 sec. 200 µL
3 min.	Incubation or linear course (0.999) Acetic acid (20%)	3 min 200 µL
△ A/min	405 nm	A

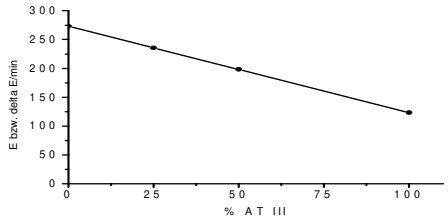
ANALYSES RESULTS

REFERENCE RANGE

0.80 – 1.20 IU AT III/mL (80 - 120% of normal)

EVALUATION USING A REFERENCE CURVE (example manual method)

To establish a reference curve 3 serial dilutions of Coagulation Reference are prepared and tested together with an optional blank (AT III Buffer A1) reading.
Predilute the Coagulation Reference 1:81 with AT III Buffer A1 (0.025 mL plasma + 2.0 mL AT III Buffer A1). From this predilution prepare a series of dilutions (1:1, 1:2, 1:4) also with AT III Buffer A1 (the 1:1 dilution corresponds to the 1:81 predilution).
This series should be tested in the same way as a sample in the assay. The absorption increases (△A/min) in the kinetic method or the absorption (A) in the end-point method are plotted on linear graph paper as readings for 100, 50 and 25% AT III value and are plotted to give a linear calibration curve. The blank reading may be used in the reference curve as the 0 % AT III value.
Example (manual method):



All samples diluted 1:81 can be read off directly from the reference curve. For dilutions other than 1:81, the % activity read off from the calibration curve has to be converted as follows:

$$\frac{\%\ AT\ III\ (reference\ curve)}{81} \times actual\ dilution\ ratio = \%\ AT\ III\ sample$$

EVALUATION USING A SEPARATE FACTOR

In this case the Coagulation Reference is dissolved in distilled water up to 100 % as indicated in the table, diluted 1:81 like a plasma sample and directly read off. The AT III activities of the samples can be calculated as follows:

$$F = \frac{100}{A_{RBR} - A_{Coag.\ Ref.\ 100\%}}$$

$$\%\ AT\ III_{sample} = (A_{RBR} - A_{sample}) \times F$$

F = Factor (calculated for each laboratory)
A = Absorption (A) or absorption increase (△A/min)
RBR = Reagent Blank Reading
Coag. Ref. = Coagulation Reference

The factor has to be calculated for each batch and/or checked for each series, both within the normal and the pathological ranges.

STANDARDISATION

The Coagulation Reference is calibrated against the International Reference Preparation of the WHO.

LIMITATION OF THE TEST

The values found when testing Coagulation Control N and Coagulation Control A should be compared to the value given on the data-key for the corresponding lot of Control plasma. If the results obtained are outside the recommended range, avoid measuring samples until the problem is solved.
A new calibration is required for each batch of reagents and for each instrument used. Also a new calibration is recommended, if software changes are introduced or following a major service of either instruments or equipment.

LITERATURE

Please contact Technoclone or your local distributor.