TEChrom AT

CE | IVD | REF | C1010-020

In-Vitro-Diagnostic reagent for professional use

Intended Use
Chromogenic test for quantitative determination of Antithrombin (AT) activity in citrated human plasma as an aid in diagnosis of Antithrombin deficiency. Intended to be used by professional laboratory personnel using coagulation analyzers measuring at 405 nm.

Background: Antithrombin (AT) is the primary physiological inhibitor of thrombin and factor Xa (FXa) in plasma and thereby effectively regulates blood coagulation. Unfractionated (UF) and low molecular weight (LMW) heparin greatly enhances AT activity. Hereditary or acquired AT deficiency is an important risk factor for venous thromboembolic disorders.[1,2] MRX Antithrombin is not affected by Heparin Cofactor II activity

Antithrombin activity is determined with two stage chromogenic method:
1. Excess of FXa is added to a citrated plasma in the presence of heparin.
2. A factor Xa-specific chromogenic substrate is added and the rate of hydrolyis is monitored with a photometer at 405nm. The release of pNA is depending on the residual factor Xa and inversely proportional to the AT activity. AT activity is expressed as %.

Contents & Determination

Product	TEChrom AT
REF.	C1010-020
Factor Xa Reagent	6 x 6mL , Bovine FXa, 10 nkat/mL, in buffer containing heparin, stabilisers and preservative.
Factor Xa Substrate	3 x 3mL, Chromogenic FXa substrate in water medium containing detergent and preservatives.
Determinations	360 Det (on Coatron-X ECO)

Recommended additional material (not included in package)

Sample dilution	A0590-125 IBS Buffer, 1 x 125mL
Calibration	P8001-005 TECal N, 5 x 1mL
Quality Control	P6001-010 TEControl N, 10 x 1mL P6101-010 TEControl A, 10 x 1mL

Preparation
Factor Xa Reagent: Ready to use. Swirl gently before use.
Factor Xa Substrate: Ready to use. Swirl gently before use.

Storage & Stability
Unopened reagents are stable until the expiration date shown on the label stored at 2°-8°C. Reconstituted reagent:

	2-8 °C	12-15 °C	37°C
Factor Xa Reagent	1 month	48 hours	4hours
Factor Xa Substrate	1 month	48 hours	4hours

Precautions
Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV and HCV. However products from human blood should be considered as potentially infectious.

Some components of this kit contain sodium azide as a preservative. To prevent the build-up of potentially explosive metallic azides in metal plumbing, flush sinks with copious amounts of water and decontaminate regularly with sodium hydroxide solution.

Specimen collection and storage[7]
Venous blood is collected in 3.2% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. Trauma or stasis during blood sampling should be avoided. Blood should not be collected through a heparin lock or other heparinised line. Inverse immediately after sampling. The presence of any clots in a specimen is a cause for rejection. Centrifuge to produce platelet-poor plasma and use for analysis. Refer to CLSI guideline H21-A5 for further instructions on specimen collection, handling and storage.[3]

Stability of plasma: 4h at 18-26°C 8h at 2-8° 14d at -20°C 6m at -70°C

Procedure
A. Automated method: Coatron A
Download application book of device from our website
https://www.teco-medical.com/downloads.html

B. Manual method: Coatron X

Pipette to empty cuvette	25 µl sample 1:11 diluted in IBS Buffer
Add	25µl Factor Xa Reagent
Incubate at 37°C	1-2min
Add	25µl Factor Xa Substrate.

Calibration Coatron X
Prepare the concentrations according to table below, run in duplicates on device and transfer manually the results to its calibration curve.

STD (%ATIII)	Plasma	IBS Buffer
100%	50µL Calibrator	500µL
50%	200µL 100% STD	200µL
25%	200µL 50% STD	200µL
12.5%	200µL 25% STD	200µL
Patient or Control	50µL Plasma	500µL

Expected Results
Expected values for antithrombin activity are typically in the 0.84 - 1.17 IU/mL range.[4] Due to inter-laboratory variation, each laboratory should establish its own reference interval.

Quality Control
TEControl or other commercially control plasma should be used for reliable quality control of performance at a frequency in accordance with good laboratory practice (GLP).

TEControl can be frozen one time after reconstitution. 120-150 µl stored in closed polypropylen tubes at -20°C is stable for 30 days

Specificity & Interferences
TEChrom AT (anti-Xa) liquid, which is based upon the use of FXa, is not influenced by Heparin Cofactor II activity. Furthermore, no influence is caused by UF and LMW heparin up to 4.2 U/mL, by bilirubin up to 43 mg/dL, by triglycerides up to 520 mg/dL and by hemoglobin up to 140 mg/dL.

Performance Characteristics
Typical performance on instrument Coatron X

Precision:

Sample	Mean AT	Repeatability CV	
TEControl N		0.99 IU/mL	3.5%
TEControl A		0.43 IU/mL	4.6%

Linearity: 0.15 – 1.25 IU/mL

Limitations
Great care must be taken to minimize variations which may occur by seemingly insignificant factors.

A. Specimen Collection. AVOID:
1. Use only plastic tubes or siliconised glass.
2. Delayed mixing of blood with anticoagulant.
3. Contamination with tissue thromboplastin.
4. Improper ratio of anticoagulant with blood.
5. Hemolyzed, icteric or lipemic samples may interfer optical systems

B. Laboratory Techniques
1. Perform tests at 37°C.
2. Use only high purity water.
3. Optimum pH is 7.0-7.5.

Warranty
This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of a foresaid express warranty.

References

1 QUINSEY, Noelene S., et al. Antithrombin: in control of coagulation. The international journal of biochemistry & cell biology, 2004, 36.3: 386-389.
2. DAHLBÄCK, Björn. Blood coagulation. The Lancet, 2000, 355.9215: 1627-1632.
3. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI document H21-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
4. KITCHEN, Steven., et al. Appendix 1 Normal ranges. Practical Hemostasis and Thrombosis 209-214 (2005)

Symbols Key:

	Expiry date	IVD	In Vitro Diagnostica		Biological hazard	REF	Catalogue Number		Consult accompanying documents
	Store at 2-8°C	CE	EU conformity		Manufacturer	LOT	Lot. Number	EC REP	Authorized Representative