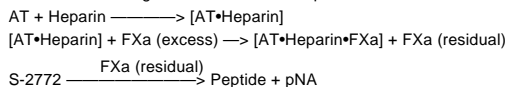


Intended use

For the quantitative determination of the heparin cofactor activity of antithrombin (AT) in human citrated plasma. All components of the kit are in liquid formulation (LR=Liquid Reagents)

Summary and principle

Antithrombin is the most important natural inhibitor of the coagulation cascade. By inhibiting the coagulation proteases, especially thrombin, factor Xa and factor IXa, antithrombin prevents uncontrolled coagulation and thrombosis. Plasma is incubated with an excess of Factor Xa (FXa) in the presence of heparin. The residual activity of FXa is determined by the rate of hydrolysis of the chromogenic substrate S-2772. The pNA release measured at 405 nm is inversely proportional to the AT level in the range 15 -125% of normal plasma.



Composition

The COAMATIC LR Antithrombin kit consists of:

- Substrate solution S-2772, 26 mg** 2 vials
Contains chromogenic substrate S-2772 (Ac-D-Arg-Gly-Arg-pNA•2HCl) in tartaric acid buffer pH 4.2.
- Factor Xa solution, 90 nkat** 6 vials
Contains purified bovine Factor Xa, bovine albumin and heparin, in Tris buffer, pH 8.2.

PRECAUTIONS AND WARNINGS:

Avoid contact with skin and eyes (S24/25).
Do not empty into drains (S29).
Wear suitable protective clothing (S36).
This product is for *in vitro* diagnostic use.

Preparation

- Substrate S-2772:** ready to use
- Factor Xa:** ready to use

Storage conditions and stability

Unopened reagents and calibrator are stable until the expiration date shown on the vial when stored at 2-8°C.

- S-2772**
Stability once opened: 6 months at 2-8°C in the original vial.
- Factor Xa**
Stability once opened 1 month at 2-8°C in the original vial.

WARNING: Do not use reagents beyond the expiry date printed on the package label. Discard if the substrate solution appears yellow. Avoid contamination by microorganisms.

Specimen collection and preparation

Nine parts of freshly drawn venous blood is collected into one part trisodium citrate. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C. Refer to NCCLS documented H21-A2 for further instructions on specimen collection, handling and storage ³.

Additional reagents and control plasmas

- Calibration plasma
- Control Plasma Abnormal Level 1&2
- Control Plasma Normal
- Saline (0.9% NaCl)

Calibration

A standard curve is obtained by analyzing different dilutions in saline of Calibration Plasma which should be traceable to the International Standard.

Calculation

The change in absorbance for the standards are plotted against the AT activity. The sample results are then calculated from the linear equation obtained from the standard curve. This procedure is automatically handled by automatic instruments.

Quality controls

Normal and abnormal controls are recommended for reliable quality control ¹. Assigned values of Controls should be traceable to the International Standard. Each laboratory should establish its own mean and standard deviation and should establish a quality program to monitor laboratory testing. Controls should be analyzed at least every 8 hours in accordance with good laboratory practice. Refer to Westgard et al for identification and resolution of out of control situations ².

Results

Antithrombin results are reported in activity (%).

Expected values

Range: 81-123% (2 SD, n=120) in a normal healthy population evaluated with COAMATIC LR Antithrombin.

Due to many variables, which may affect results, each laboratory should establish its own normal range.

Procedures

All conditions included in this package insert are referred to ACL Futura. Detailed instrument settings including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix.

*NOTE: Not all instrument applications are available in all countries.

Performance Characteristics

Limitations/ interfering substances

Most functional AT assays are based on the capacity of AT in plasma to inactivate exogenously added thrombin in the presence of heparin ¹. However, heparin cofactor II present in the plasma may also inhibit the added thrombin and lead to overestimation of AT activity ². Consequently, it has been shown that a FXa-based AT assay discriminates better between AT-deficient and non-AT deficient individuals than a thrombin based assay ³. It also allows for accurate AT determination in patients who are receiving heparin therapy ⁴. Coamatic LR AT is based on inhibition of FXa, thus eliminating the risk of contribution from heparin cofactor II.

AT results are not affected by Triglycerides at concentrations of 850 mg/dL, Bilirubin at concentrations of 38 mg/dL and Hemoglobin at concentrations of 275 mg/dL

Precision:

Within and between run precision was assessed over multiple runs.

ACL Futura Mean (% AT)	CV% (Within run)	n	CV% (Between run Total)	n
114	1.96	6	3.52	60
59	6.21	6	6.43	60
29	8.45	6	11.1	60

Correlation:

System	slope	intercept	r	Reference method
ACL Futura	1.011	0.4889	0.995	IL Test Liquid AT on ACL 9000

Linearity:

System

ACL Futura 15 – 125% Antithrombin

Sample results above 125% should be manually diluted 1:2 and re-assayed. The printed results must be multiplied by 2 to correct for the dilution.

Detection Limity:

System

ACL Futura 15% Antithrombin

Sensitivity:

System

ACL Futura mAbs / min per 1% Antithrombin activity: 3.7 / min






Determinations/kit

On ACL Futura 440 tests (approximately)

Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia / Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

- ZUCKER S, CATHEY M H, WEST B. Preparation of Quality control specimens for coagulation. Am J Clin Pathol 53, 924-927 (1970).
- WESTGARD J O, BARRY P L. Cost-effective quality control: Managing the quality and productivity of analytical process. AACC press (1988).
- National Committee for Clinical Laboratory Standards. Collection, transport and processing of blood specimens for coagulation testing and performance of coagulation assays, NCCLS Document H21-A2; vol 11 No. 23.
- ÖDEGÅRD O R et al. Heparin cofactor activity measured with an amidolytic method. Thromb Res 6, 287-294 (1975).
- TRAN H et al. Influence of heparin cofactor II (HCII) in the determination of antithrombin III (AT). Thromb Res 40, 571-576 (1985).
- TOLLEFSEN D M. Laboratory diagnosis of antithrombin and heparin cofactor II deficiency. Semin inThromb Hemost 16, 162-168 (1990).
- DEMERS C et al. An antithrombin III assay based on factor Xa inhibition provides a more reliable test to identify congenital antithrombin III deficiency than an assay based on thrombin inhibition. Thromb Haemost 69, 231-235 (1993).
- BOHNER J et al. Thrombin-based antithrombin assays show overestimation of antithrombin III activity in patients on heparin therapy due to heparin cofactor II influence. Thromb Haemost 71, 280-283 (1994).

Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Συμβολημένοι / Συμμοποιηθέντα σύμβολα

IVD	LOT				CONTROL			EC REP
<i>In vitro</i> diagnostic medical device <i>In-vitro</i> Diagnostikum De uso diagnóstico <i>in vitro</i> Dispositif médical de diagnostic <i>in vitro</i> Per uso diagnostico <i>in vitro</i> Dispositivo médico para utilização em diagnóstico <i>in vitro</i> "in vitro" diagnostisk udstyr <i>In vitro</i> diagnostisk medicinsk produkt Προϊόν για διαγνωστική χρήση <i>In vitro</i>	Batch code Chargen-Bezeichnung Identificación número de lote Désignation du lot Numero del lotto Número de lote Batch nr. Tillverkningskod Αρ. Παρτίδας	Use by Verwendbar bis Caducidad Utilisable jusqu'à Da utilizzare prima del Data límite de utilização Anvendelse Användning Χρήση έως	Temperature limitation Festgelegte Temperatur Temperatura de Almacenamiento Températures limites de conservation Limiti di temperatura Limite de temperatura Temperatur begrænsninger Temperatur gräns Περιορισμοί θερμοκρασίας	Consult instructions for use Beilage beachten Consultar la metódica Lire le mode d'emploi Vedere istruzioni per l'uso Consultar as instruções de utilização Se vejledning for anvendelse Ta del av instruktionerna före användning Συμβουλευτείτε τις οδηγίες χρήσης	Control Kontrollen Control Contrôle Controllo Controlo Kontrol Kontroll Υλικό ποιοτικού ελέγχου	Biological risks Biologisches Risiko Riesgo biológico Risque biologique Rischio biologico Risco biológico Miljø oplysninger Biologiska risker Βιολογικοί κίνδυνοι	Manufacturer Hergestellt von Fabricado por Fabricant Prodotto da Fabricado por Producent Tillverkare Κατασκευαστής	Authorised representative Bevollmächtigter Representante autorizado Mandataire Rappresentanza autorizzata Representante autorizado Leverandør Auktoriserad representant Εξουσιοδοτημένος αντιπρόσωπος