

### Intended use

For the quantitative determination of the heparin cofactor activity of antithrombin (AT) in human citrated plasma.

### 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 10-125% of normal plasma.

AT + Heparin → [AT•Heparin]

[AT•Heparin] + FXa (excess) → [AT•Heparin•FXa] + FXa (residual)

FXa (residual)

S-2772 → Peptide + pNA

### Composition

The COAMATIC AT 400 kit consists of:

- Substrate S-2772, 26 mg** 2 vials  
Lyophilized chromogenic substrate (Ac-D-Arg-Gly-Arg-pNA•2HCl).
- Buffer with heparin, 25 mL** 6 vials  
This buffer, pH 8.2, ionic strength 0.25.
- Factor Xa, 90 nkat** 6 vials  
Contains purified bovine Factor Xa and bovine albumin.

### PRECAUTIONS AND WARNINGS:

Harmful if swallowed (R 22).  
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

The reagents are reconstituted according to the specific instrument application.  
For ACL Futura:

- Substrate S-2772:** reconstitute with 20 mL of sterile water <sup>1</sup>
- Factor Xa :** reconstitute with 10 mL of heparin buffer

After reconstitution replace the stoppers and swirl gently. Make sure of the complete reconstitution of the product. Keep reagents at 15-25°C for 10-30 min and invert before use.

NOTE: Other reagent reconstitution volumes may apply for other automated methods. The reagents are not interchangeable between lots.

### Reagent storage and stability

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

- Substrate S-2772: Stability after reconstitution 6 months at 2-8°C in the original vial.
- Buffer with heparin: Stability when opened 3 months at 2-8°C in the original vial.
- Factor Xa Stability after reconstitution 3 months 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.<sup>4</sup>

### Additional reagents and control plasmas

- Deionized water, filtered through 0.22 mm or NCCLS type II water.<sup>1</sup>
- Calibration plasma
- Control Plasma Abnormal
- Control Plasma Normal
- Saline (0.9% NaCl)

### Quality control

Normal and abnormal controls are recommended for reliable quality control.<sup>2</sup> 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.<sup>3</sup>

### Results

Antithrombin results are reported in activity (%).

### Expected values

82 - 124% (2 SD, n=122) in a normal healthy population evaluated with Coamatic AT 400. 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.

### 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.

### 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.<sup>5</sup> However, heparin cofactor II present in the plasma may also inhibit the added thrombin and lead to overestimation of AT activity.<sup>5,7</sup> 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.<sup>8</sup> It also allows for accurate AT determination in patients who are receiving heparin therapy.<sup>9</sup> Coamatic AT 400 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 900 mg/dL, Bilirubin at concentrations of 38 mg/dL and Hemoglobin at concentrations of 619 mg/dL.

#### Precision:

Within run and total precision was assessed over multiple runs.

ACL Futura	CV% (Within run)	n	CV% (Total)	n
Mean (%AT)				
113	1.80	6	2.81	60
59	2.64	6	3.23	60
31	4.52	6	4.76	60

#### Correlation:

System	Slope	Intercept	r	Reference method
ACL Futura	0.9810	4.9773	0.995	IL Test Antithrombin on ACL 9000

#### Linearity:

##### System

ACL Futura 10 - 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 Limit:

##### System

ACL Futura: 10 % Antithrombin

#### Sensitivity:

##### System

ACL Futura mAbs / min for 1% Antithrombin activity: 8.2 / min






### Determinations/kit

On ACL Futura 420 tests (approximately)

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

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8. 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).
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## Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Συμβοληθθέντα 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 instruktionen 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