

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, AT prevents uncontrolled coagulation and thrombosis.

Plasma is incubated with an excess of factor Xa (FXa) in the presence of heparin. The residual quantity of FXa is determined by the rate of hydrolysis of the chromogenic substrate S-2765. The pNA release measured at 405 nm is inversely proportional to the AT level in the range from 0-120% of normal plasma.¹

1. AT + Heparin → [AT · Heparin]
2. [AT · Heparin] + FXa (excess) → [AT · Heparin · FXa] + FXa (residual)
3. S-2765 $\xrightarrow{\text{FXa (residual)}}$ Peptide + pNA

Composition

1. **S-2765, 8 mg** 1 vial
Lyophilized chromogenic substrate N- α -Cbo-D-Arg-Gly-Arg-pNA-2HCl.
2. **Buffer with heparin, 25 mL** 1 vial
Tris buffer, pH 8.2, ionic strength 0.25.
3. **Factor Xa, 29 nkat** 1 vial
Lyophilized purified bovine Factor Xa and bovine albumin.

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

The reagents are reconstituted according to the specific instrument application. For microplate and test tube techniques:

1. **Substrate S-2765:** reconstitute with 10 mL of NCCLS Type II water or equivalent.
2. **Factor Xa:** reconstitute with 10 mL of buffer with heparin. Replace the stoppers and swirl gently. Make sure of the complete reconstitution of the product. Keep reagent at 15-25°C for 10-30 min and invert before use.

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

Storage conditions and stability

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

1. **S-2765:** Stability after reconstitution: 6 months at 2-8°C in the original vial.
2. **Buffer:** with heparin Stability when opened: 3 months at 2-8°C in the original vial.
3. **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 are collected into one part trisodium citrate. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C.

Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage.⁵

Additional reagents and control plasmas

1. Deionized water, filtered through 0.22 mm or NCCLS type II water.²
2. Calibration plasma
3. Control Plasma Abnormal and Normal
4. Saline (0.9% NaCl).
5. Acetic acid 20% or citric acid 2% (end-point methods).

Materials required but not provided

- Spectrophotometer, 405 nm (and 490 nm for microplate procedure)
- Microplate* or semi-micro cuvettes (1 cm)
- Centrifuge, 2000 x g
- Incubator 37°C \pm 0.2°C
- Vortex mixer
- Stopwatch
- Calibrated pipettes
- Plastic test tubes

*NOTE: Do not use microplates intended for coating!

Quality controls

Normal and abnormal controls are recommended for a complete quality control program.³ 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 control program to monitor laboratory testing. Controls should be analyzed at least every 8 hours in accordance with good laboratory practice. Refer to Westgard et al⁴ for identification and resolution for out of control situations.

Results

Antithrombin results are reported in activity (%).

Expected values

106 \pm 18% (2 SD, n=109 ; M=53 F=56) in a normal healthy population evaluated with Coamatic Antithrombin. Due to many variables which may affect results, each laboratory should establish its own normal range.

Procedures

All conditions and performance characteristics included in this package insert are referred to microplate method. 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, to obtain antithrombin levels of 0, 25, 50, 75 and 100%. A standard about of 120% can be prepared by diluting 25 mL Calibration Plasma with 2500 mL of saline. The assigned value of calibrator plasma should be traceable to the International Standard.

Assay conditions for microplate and test tube techniques:

Dilution of samples and controls

Samples/controls/standards	25 μ L
Saline	3000 μ L
Mix well	

Microplate method

Diluted samples/controls/standards	50 μ L
Factor Xa	50 μ L
Incubate at 37°C, 90 sec	
S-2765 (pre-heat at 37°C)	50 μ L
A. Kinetic method: read DA/min at 405 nm for 30-90 sec.	
B. End-point method: proceed as described below.	
Incubate at 37°C, 90 sec	
Acetic acid 20% or 2% citric acid	50 μ L

Mix

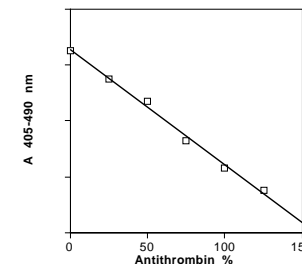
Read the absorbance against water at 405 nm. If possible, read and subtract the absorbance at 490 nm in order to compensate for differences in the material of the microplate wells.

Test tube method

Use 200 μ L instead of 50 μ L for all pipetting steps.

Calculation

Plot the change in absorbance per minute (ΔA /min) or absorbance (A) for the standards against their antithrombin activity on linear graph paper. Plot ΔA /min or A on the Y axis and % antithrombin on the X axis. Connect the standard points with the best fitting straight line. Samples are evaluated based on this standard curve. An example of a typical standard curve (microplate method) is shown below.



Performance Characteristics

Limitations/interfering substances

No drug interference reported. Since the method is based on inhibition of factor Xa there is no influence from heparin cofactor II, α_2 -macroglobulin or α_1 -antitrypsin.^{6,7,8} In plasmas where contact activation has occurred, a contribution to the substrate activity might be produced. Thus an underestimation of the antithrombin level might follow. To improve the validity of the assay the value obtained in absence of factor Xa can be subtracted from the sample value. Bilirubin, haemoglobin and plasma from hyperlipaemic patients interfere in absorbance readings. In these cases individual plasma blanks are necessary when acid stopped methods are used.

Precision:

Microplate method	CV% (Within day)	n	CV% (Between days)	n
Mean (%AT)				
100	3.1	20	2.6	5
50	4.8	20	4.3	5

Correlation:

The assay has been compared with Coatest Antithrombin and Coacutec Antithrombin R using normal plasmas, patient plasmas and mixtures of normal and deficiency plasmas.

System	Slope	Intercept	r	Reference method	n
Microplate	1.03	-2.6	0.97	Coatest	45
ACL	1.01	-1.6	0.96	Coatest	43
Microplate	1.13	-7.5	0.99	Coacutec	43
ACL	0.95	2.3	0.99	Coacutec	43

The precision and correlation results were obtained using specific lots of reagents and controls.

Linearity:

System

Microplate 0 - 120% Antithrombin

Detection Limit:

System

Microplate: 5 % Antithrombin

Sensitivity:

System

Microplate mAbs / min per 1% Antithrombin activity : 7.9 / min






Determinations/kit

Microplate method: 200 Test Tube: 50

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

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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα 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 Límite 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 Συμβουλευτήτε 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