**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 inactivating 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 pH value released at 405 nm is inversely proportional to the AT level in the range from 0-100% of normal plasma.

1. AT + Heparin → [AT · Heparin]
2. [AT · Heparin] + FXa or FXa(excess) → [AT · Heparin] · FXa or FXa (residual)
3. S-2765 → Peptide + pNA

**Composition**

1. S-2765, 8 mg 1 vial
   - Lyophilized chromogenic substrate Na2-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).

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. Buffer: reconstitute with 10 mL of buffer with heparin.
3. Factor Xa: reconstitute with 10 mL of buffer with heparin.

Storage conditions and stability

- **Drop out reagents are stable until the expiration date shown on the vial when stored at 2-8°C.**
- **S-2765**: Stability after reconstitution: 6 months at 2-8°C in the original vial.
- **Buffer**: Stability after opening: 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.

**Specimen collection and preparation**

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate.

**Additional reagents and control plasmas**

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

**Materials required but not provided**

- Spectrophotometer, 405 nm (and 490 nm for microplate procedure)
- Microplate® or semi-micro cuvettes (1 cm)
- Centrifuge, 2000 g
- Incubator 37°C ± 0.2°C
- Vortex mixer
- Stopwatch
- Calibrated pipettes

**Quality controls**

Normal and abnormal controls are recommended for a complete quality control program. 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 al.1 for identification and resolution for out of control situations.

**Results**

Antithrombin results are reported in activity (%).

**Expected values**

- TOXICITY: <400 activity units/mL; m:10S; M:+0.5 (see table) in a normal healthy population evaluated with Chromogenic Antithrombin. Due to many variables which may affect results, each laboratory should establish its own normal range.

**Procedures**

Assay conditions for microplate and test tube techniques:

- Dilution of samples and controls
  - Samples/controls/standards 25 µL
  - Saline 3000 µL
  - Mix well
- Microplate method
  - Diluted samples/controls/standards 50 µL
  - Factor Xa 50 µL
  - S-2765 (pre-heated at 37°C)
  - Incubate at 37°C, 90 sec
- A. Kinetic method: read DA/min at 405 nm for 90-120 sec.
  - B. End-point method: proceed as described below.
  - Incubate at 37°C, 90 sec
  - Acidic acid 20% or 2% citric acid 2% 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 µL instead of 50 µL for all pipetting steps.

**Materials and interfering substances**

- Microplate method.
  - Linearity:
    - System
    - Microplate Slope Intercept r Reference method n
    - ACL 1.03 -1.6 0.96 Coatest 43
    - Microplate 1.03 -2.6 0.97 Coatest 45
  - Calibration:
    - System
    - Microplate Slope Intercept r Reference method n
    - ACL 1.03 -1.6 0.96 Coatest 43
    - Microplate 1.03 -2.6 0.97 Coatest 45
  - Precision:
    - System
    - Microplate Slope Intercept r Reference method n
    - ACL 1.03 -1.6 0.96 Coatest 43
    - Microplate 1.03 -2.6 0.97 Coatest 45
  - Correlation:
    - System
    - Microplate Slope Intercept r Reference method n
    - ACL 1.03 -1.6 0.96 Coatest 43
    - Microplate 1.03 -2.6 0.97 Coatest 45

**Performance Characteristics**

- 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.

**Calculation**

Plot the change in absorbance per minute (µA/min) or absorbance (A) for the standards against the factor Xa activity on linear graph paper. Plot µA/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.

**References**

1. Westgard et al.2

**ENGLISH - Insert revision 07/2002**
5. 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.