CORMAY ANTITHROMBIN III

DIAGNOSTIC KIT FOR DETERMINATION OF ANTITHROMBIN III CONCENTRATION

Kit name	Kit size	Cat. No
CORMAY ANTITHROMBIN III	1 x 58.5 ml	4-592

INTRODUCTION

Antithrombin III (AT III) is an α2-glycoprotein of MW 58000 and is made in the liver. AT III is one of the most important regulators of the coagulation system. AT III inactivates thrombin and factors Xa, IXa, XIa and XIIa. This anticoagulant activity is enhanced by the presence of heparin, which forms a ternary complex with AT III and these procoagulant factors. Reduced concentration of AT III in blood means a great and well established risk for thrombotic complications. Clinical low value are associated with congenital or acquired deficiencies caused by a decreased biosynthesis (liver disease, medical treatment) or an increased loss (gastrointestinal disease, nephrotic syndrome) or an increased consumption (sepsis, major trauma due to surgery and burns wounds).

METHOD PRINCIPLE

The antithrombin III presents in a sample form with the specific antibody an immunological complex. The increase of turbidity after the addition of antiserum measured at λ=340 nm is proportional to antithrombin III concentration in the sample.

REAGENTS

Package

1-Reagent	1 x 48.5 ml
2-Reagent	1 x 10 ml

Buffer (1-Reagent) stored at 2-25°C and antiserum (2-Reagent) stored at 2-8°C are stable until expiry date printed on the package. Store closed. Protect from light and contamination!

Concentrations in the test

Tricine buffer (pH 8.0); PEG; sodium chloride; anti human antithrombin III antiserum; HEPES buffer (pH 7.4); sodium azide (< 1 g/l); stabilizers.

Warnings and notes

- Products for in vitro diagnostic use only.
- The reagents must be used only for the intended purpose, by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Products from human source have been tested for HBsAg and antibodies to HIV and HCV and found to be non-reactive. However this material should be handled as thought capable of transmitting infectious disease.
- Products contain sodium azide (< 1 g/l) as a preservative. Avoid contact with skin and mucous membranes.

ADDITIONAL EQUIPMENT

- automated clinical chemistry analyser capable of accommodating two-reagent assays or photometer able to read at 340 nm;
- general laboratory equipment;

SPECIMEN

Citrated plasma.

Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

The reagents are ready to use.

These reagents may be used for manual assay and in automatic analysers according to their user manual. Applications for analysers are available on request.

These reagents may be used directly in Hitachi 911/912 analysers.

Application should be entered using handheld barcode scanner and attached barcodes sheet, according to procedure described below:

1. Delete previous version of application and calibrators assigned to it and restart the analyser.
2. Enter codes of calibrators according to the attached list.
3. Enter barcoded application and assign proper values to calibrators.
4. To activate entered application go to the tab UTILITY | APPLICATION | RANGE and change value of field DATA MODE from INACTIVE to ON BOARD. Confirm the change using UPDATE button.
5. Put reagents on board the analyser – they will be assigned to relevant tests automatically. Perform also measurement of level of reagents inside the bottles.
6. After calibration analyser is ready to use.

Manual procedure

wavelength	340 nm
temperature	37°C

For calibration kit with five calibrators which contain antithrombin III at various concentrations CORMAY IMMUNO-MULTICAL (Cat. No 4-287) is recommended. Alternatively there is one point calibration – with a calibrator ≤ 0.40 g/l; in this case samples with levels above 0.50 g/l should be reassayed after 10x dilution in 0.9% NaCl.

The calibrator, the controls and unknown samples must not be diluted.

Pipette into the cuvettes:

(Volumes could be modified by respecting the reagent: sample ratio.)

	blank (B)	calibrator (C)	test (T)
1-Reagent	250 µl	250 µl	250 µl
sample	-	-	10 µl
calibrator	-	10 µl	-
deionized water	10 µl	-	-

Mix well, after 5 min. of incubation at 37°C read the absorbance A_1 of calibrator (C), test samples (T) and blank (B) against water or air.

Then add:

2-Reagent	50 µl	50 µl	50 µl

Mix well, after exactly 5 min. of incubation at 37°C read the absorbance A_2 of calibrator (C), test samples (T) and blank (B) against water or air.

Calculation

Calculate the increase of absorbance:

$\Delta A(T) = (A_2 - A_1)T - (A_2 - A_1)B$

$\Delta A(C) = (A_2 - A_1)C - (A_2 - A_1)B$

antithrombin III concentration = $\Delta A(T)/\Delta A(C)$ x calibrator concentration

REFERENCE VALUES [7]

plasma	0.25 – 0.45 g/l

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
For internal quality control it is recommended to use the CORMAY IMMUNO-CONTROL III (Cat. No 4-291) with each batch of samples.
For the calibration of automatic analysers systems the CORMAY IMMUNO-MULTICAL (Cat. No 4-287) is recommended. **Calibrators and 0.9% NaCl** should be used for calibration.
The calibration curve should be prepared, every 4 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
These metrological characteristics have been obtained using the automatic analysers Hitachi 912 and Cobas Mira. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.052 g/l.

- **Linearity:** up to 0.7 g/l.

- **Specificity / Interferences**

Hemoglobin up to 0.32 g/dl, bilirubin up to 29.5 mg/dl, triglycerides up to 1000 mg/dl, heparin up to 0.5 g/l, sodium fluoride up to 4 g/l, EDTA up to 5 g/l, sodium citrate up to 5 g/l do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	17.0	0.6	3.51
level 2	40.0	1.0	2.47

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	19.5	0.4	2.2
level 2	31.4	0.8	2.5

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

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MANUFACTURER

PZ CORMAY S.A.
ul. Wiosenna 22,
05-092 Łomianki, POLAND
tel.: +48 (0) 22 751 79 10
fax: +48 (0) 22 751 79 14
http://www.pzcormay.pl

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