

Dia-AT III (FIIa)

ANTITHROMBIN III CHROMOGENIC ASSAY



Cat. No.: 33012 **4 x 3 ml Thrombin**
4 x 3 ml Substrate
4 x 7 ml Diluent

PRODUCT NAME

Dia-AT III (FIIa) chromogenic assay.

INTENDED USE

(For In Vitro Diagnostic Use Only)

Dia-AT III (FIIa) is a chromogenic assay used for the quantitative determination of Antithrombin III (AT III) activity in human citrated plasma.

SUMMARY AND EXPLANATIONS

AT III is an irreversible inhibitor of thrombin (FIIa) and FXa. Acquired or hereditary AT III deficiency is an important risk factor of thrombosis.

AT III and thrombin form a stoichiometric complex. The velocity of the complex formation is highly increased by the presence of heparin, thus the antithrombotic effect appears immediately when heparin is present.

PRINCIPLE

Dia-AT III (FIIa) assay for determination of the AT III level consists of two steps:

1. The plasma sample is incubated with a known, excess amount of thrombin in the presence of heparin.
2. After the complex formation, the activity of the residual thrombin is determined by a chromogenic thrombin substrate (absorbance measurement at 405nm). The amount of the inhibited thrombin is proportional to AT III level of the sample.

ACTIVE INGREDIENTS

- Dia-AT III (FIIa) Thrombin reagent is a freeze-dried bovine thrombin in buffered medium which contains heparin and preservative.
- Dia-AT III (FIIa) Substrate is a freeze-dried chromogenic thrombin substrate which contains preservative.
- Dia-AT III (FIIa) Diluent is a buffer which contains preservative.

PRECAUTIONS

- Person installing the Dia-AT III (FIIa) assay must be a trained laboratory professional!
- Dia-AT III (FIIa) assay's reagents, due to its ingredients should be handled with care by observing the precautions recommended for biohazards material!
- Reagent coming into contact with specimens and other materials should be handled as if

capable of transmitting infection and should be disposed of with proper precautions!

- Avoid microbial contamination of the reagent otherwise erroneous results may occur!
- According to the present knowledge the reagent does not contain any particles which can spread from animal to human!
- All reagents, waste and utilized disposable laboratory equipment should be considered as hazardous waste! Their handling and disposal should be done according to the valid hazardous material processing regulation.
- Do not use the reagent beyond the expiration date printed on the label!

PREPARATION

Dia-AT III (FIIa) Thrombin and Substrate reagents are dissolved with 3ml distilled water. Keep the reagent at room temperature (20-25°C) for at least 30 minutes for proper reconstitution. Swirl the vial gently, horizontally more times (5-10) before using it, but do not shake.

Dia-AT III (FIIa) Diluent is ready for use.

Wait until the reagents reach the working temperature!

SPECIMENS

Dia-AT III (FIIa) assay requires freshly decalcified plasma. To obtain it, mix nine parts of freshly drawn venous blood with one part trisodium citrate (3,2%; 109mmol/L). The use of higher concentration of trisodium citrate (3,8%; 129mmol/L) is not recommended. Mix the blood carefully and centrifuge plasma before testing. Refer to Clinical and Laboratory Standards Institute (CLSI) guidelines H21-A5.

T (°C)	20-25	2-8	<-20
Plasma stability	4 hours	Unacceptable	1 month

CALIBRATION PROCEDURE

Dia-AT III (FIIa) assay calibration is a calibrator dilution based process, which can be used with semi-automated coagulation analysers (Coag 4D) according to the protocol detailed below. The duplicated measurement is recommended.

1.	Preparing 4 different Calibrator dilutions with Diluent buffer (1:20; 1:40; 1:60; Diluent)	
2.	Adding Calibrator dilution into cuvette	50µl
3.	Adding Thrombin reagent into cuvette	50µl
4.	Calibrator and reagent incubation	2min
5.	Adding Substrate reagent into cuvette	50µl
6.	Simultaneously start the reading of absorbance (OD/min)	10-40sec
7.	Preparing calibration curve from the results above (OD/min and relevant % derived from Calibrator value according to dilution)	

TEST PROCEDURE

Dia-AT III (FIIa) assay is a chromogenic test, which can be used with semi-automated coagulation analysers (Coag 4D) according to the protocol detailed below. The duplicated measurement is recommended.

1.	Sample dilution with Diluent buffer	1:20
2.	Adding diluted sample into cuvette	50µl
3.	Adding Thrombin reagent into cuvette	50µl
4.	Sample and reagent incubation	2min
5.	Adding Substrate reagent into cuvette	50µl
6.	Simultaneously start the reading of absorbance (OD/min) on 405nm	10-40sec

Normal and pathological controls are recommended for verified measuring. Each laboratory should establish its own quality control program. In case of determination by any other coagulometer, please follow the instructions of the manual.

STORAGE AND STABILITY

Dia-AT III (FIIa) assay's reagents in intact vial are stable until the expiration date given on the vial, when stored at 2-8°C. Stability after opening in the original vial are shown in below table:

T (°C)	20-25	15-19	2-8
Thrombin, Substrate (Day)	-	3	7
Diluent (Day)	3	-	7

Do not freeze them! Keep the Substrate in dark!

EXPECTED RESULTS

Dia-AT III (FIIa) assay results can be reported in percentage (%). This dimension is calculated from a lin-lin calibration curve. Each laboratory should prepare a lot specific calibration curve according to the above description (Coag 4D). In case of determination by any other coagulometer, please follow the instructions of the manual.

The normal range expressed in % in the adult population is 80-120%. Every laboratory should determine its own normal or reference range.

LIMITATIONS

Dia-AT III (FIIa) assay cannot be applied in case of the presence of thrombin inhibitors (e.g. hirudin, dabigatran, etc.) in the patient sample.

PERFORMANCE CHARACTERISTICS

The reproducibility test of Dia-AT III (FIIa) assay on Diagon analysers (Coag Line) gives the following results:

Sample	Intra-Assay		Inter-Assay	
	1	2	3	4
n	10	10	10	10
Mean (%)	105,0	45,4	104,6	45,1
CV (%)	1,982	3,954	2,360	2,998
















MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrator for the calibration (Dia-CAL; Cat. No.: 95012)
- Different levels of control for quality control (Dia-CONT I-II; Cat. No.: 91020, 91010).
- Optical or mechanical coagulation or other analyser for measuring absorbance at 405nm, Diagon analysers (Coag Line) are recommended.

BIBLIOGRAPHY

1. CLSI: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline- Fifth Edition. CLSI document: H21-A5; 28:5; 2008.
2. Blombäck M, Blombäck B, Olsson P and Svendsen L: The Assays of Antithrombin Using a Synthetic Chromogenic Substrate for Thrombin. Thromb Res; 5: 621-632; 1974.
3. Khor B, Van Cott EM: Laboratory tests for antithrombin deficiency. Am J Hematol; 85: 947-950; 2010.
4. Meijer P, Haverkate F, Kluft C: Performance goals for the laboratory testing of antithrombin, protein C and protein S. Thromb Haemost; 96: 584-589; 2006.

DIAGON LTD.
 Baross u. 48-52, 1047 Budapest, Hungary
 Tel.: +36 1 3696500
 Fax.: +36 1 3696301
 Web: www.diagon.com
 E-mail: diagon@diagon.com

SYMBOLS			
	Manufacturer		Use-by date
	Batch code		Catalogue number
	Do not use if package is damaged		Fragile, handle with care
	Keep dry		Temperature limit
	Biological risks		Consult instruction for use
	Caution		<i>In vitro</i> diagnostic medical device
	Contains sufficient for <n> tests		This side up
	CE mark		