HORIBA Medical

INSTRUCTION FOR USE

CE

Yumizen G AT

ANTITHROMBIN CHROMOGENIC ASSAY

Cat. No.: 1300036390 **4 x 3 ml Thrombin**
4 x 3 ml Substrate
4 x 7 ml Diluent

PRODUCT NAME

Yumizen G AT chromogenic assay.

INTENDED USE

(For In Vitro Diagnostic Use Only)

Yumizen G AT is a chromogenic assay used for the quantitative determination of Antithrombin (AT) activity in human citrated plasma.

SUMMARY AND EXPLANATIONS

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

AT 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

Yumizen G AT assay for determination of the AT 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 level of the sample.

ACTIVE INGREDIENTS

- Yumizen G AT Thrombin reagent is a freeze-dried bovine thrombin in buffered medium which contains heparin and preservative.
- Yumizen G AT Substrate is a freeze-dried chromogenic thrombin substrate which contains preservative.
- Yumizen G AT Diluent is a buffer which contains preservative.

PRECAUTIONS

- Person installing the Yumizen G AT assay must be a trained laboratory professional!
- Yumizen G AT 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!
- This reagent is obtained from substances of animal origin. Consequently, it should be treated as potentially infectious and handled with the appropriate cautions in accordance with good laboratory practices (5).
- 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

Yumizen G AT 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.

Yumizen G AT Diluent is ready for use.

Wait until the reagents reach the working temperature!

SPECIMENS

Yumizen G AT 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

Yumizen G AT assay calibration is a calibrator dilution based process, which can be used with semi-automated coagulation analysers (Yumizen G Line) according to the protocol detailed below.

Prepare a serial dilution of the calibrator Yumizen G CAL according to the table below:

	Cal. Point 1	Cal. Point 2	Cal. Point 3	Cal. Point 4
Dil. rate	1/20	1/40	1/60	0
Yumizen G CAL	20 µL	100 µL from Cal point 1 (1/20)	100 µL from Cal point 1 (1/20)	0 µL
Yumizen G AT Diluent	380 µL	100 µL	200 µL	100 µL
Total Volume	400 µL	200 µL	300 µL	100 µL

A duplicate measurement is recommended.

1. Add 50 µL of diluted calibrator into the cuvette.
2. Add 50 µL of Yumizen G AT Thrombin into the cuvette.

3. Incubate for 2 min.
4. Add 50µL of Yumizen G AT Substrate.
5. Start the absorbance measurement.
6. Use the results to prepare the calibration curve according to the table below.

Dilution rate	%	OD/min
1/20	X*	Result 1
1/40	X/2	Result 2
1/60	X/3	Result 3
0	0	Result 4

*** Yumizen G CAL value from the enclosed annex.**

7. Select Calibration > Chromogenic test > AT(III) > Reagent > Reagent 1.
8. Enter Name, Lot number and Expiration date.
9. Press validate (✓).
10. Select Reagent 2.
11. Enter Name, Lot number and Expiration date.
12. Press validate (✓).
13. If necessary, press **Yes** to overwrite the previous settings.
14. Press **%**.
15. Select Calibrator and enter Name, Lot number and Expiration date.
16. Enter the point pairs and validate (✓) after each point.
17. If necessary, press **Yes** to store the settings.

TEST PROCEDURE

Yumizen G AT assay is a chromogenic test, which can be used with semi-automated coagulation analysers (Yumizen G Line) according to the protocol detailed below. A duplicate 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) at 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

Yumizen G AT 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

Yumizen G AT 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 (Yumizen G Line). 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

Yumizen G AT assay cannot be applied in case of the presence of thrombin inhibitors (e.g. hirudin, dagibatran, etc.) in the patient sample.

PERFORMANCE CHARACTERISTICS

The reproducibility test of Yumizen G AT assay on Horiba Medical analysers (Yumizen G Line) gives the following results:

	Intra-Assay		Inter-Assay	
Sample	**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[a]

- Calibrator for the calibration (Yumizen G CAL; Cat. No.: 1300036416)
- Different levels of control for quality control (Yumizen G CTRL I & II control; Cat. No.: 1300036412).
- This reagent may be performed using manual, semi-automated and automated methods.
- Coagulation or other analyser for measuring absorbance at 405nm, Horiba analysers (Yumizen G Line) are recommended.

BIBLIOGRAPHY

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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-

[a]Modification: modification of materials required.

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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.
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