This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Notification No. 13A2X00197218081

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*Revised: April 2008 (9th edition)

This package insert must be read carefully prior to use.

Heparin assay kit
(Classification No.: 30564000)

Testzym HeparinS

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components) **

Component Ingredients
(1) Substrate: N-Benzoyl-L-isoleucyl-L-glutamyl (γ-OR)-glycyl-L-arginyl-p-nitroanilide hydrochloride (S-2222)
(2) Antithrombin III: Antithrombin III (human origin)
(3) Factor Xa: Factor Xa (bovine origin)
(4) Buffer Solution: 2-Amino-2-hydroxymethyl-1,3-propanediol buffer
(5) Normal Plasma: Normal human plasma

Intended Use

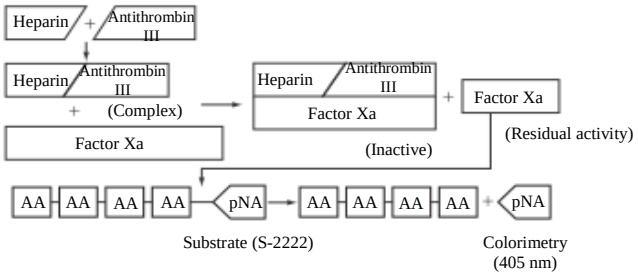
Measurement of heparin in plasma

It is known that heparin binds to antithrombin III in the blood to form a complex, which in turn binds to activated coagulation factors (such as thrombin and Factor Xa) and inhibits their activity.
Because of its action, heparin is widely used in clinical practice for prevention and treatment of disseminated intravascular coagulation (DIC) and thrombosis, as well as for anticoagulation during extracorporeal circulation. Measurement of heparin is useful for monitoring the effect of heparin therapy.

Assay Principle

1. Assay Principle

Add antithrombin III to the test plasma containing heparin to form heparin/antithrombin III complexes. When a fixed excess of Factor Xa is subsequently added to the sample, the heparin/antithrombin III complexes bind to Factor Xa, forming inactive complexes (heparin/antithrombin III/Factor Xa).
When the Substrate (S-2222) is added to the test plasma, p-nitroaniline corresponding to the residual activity of Factor Xa releases. Because the residual activity Factor Xa is related to the heparin concentration in the test plasma, the heparin concentration can be determined through measurement of the released p-nitroaniline by colorimetry at 405 nm.



2. Features

1) Heparin can be measured without any influence of other coagulation factors.
2) Handling is easy, and results can be obtained rapidly.
3) This product shows good quantification and excellent reproducibility.

Procedural Precautions **

1. Properties of Samples and Sampling Methods

1) Samples
(1)Plasma (Citrated plasma) may be used.
(2)Heparin plasma or EDTA plasma should not be used.
2) Storage of samples
(1) If the isolated plasma sample cannot be tested on the same day, specimens should be stored as follows:
2–10°C: for tests within 1 week
≤ -20°C : for tests within 1 month
Bring samples to room temperature (15–30°C) before use.
(2) Avoid repeated freezing and thawing, or errors in the assay results may occur.
(3) Use plastic containers and test tubes for dilution of plasma.

2. Interfering substances

Assay results are not affected by bilirubin (up to 20 mg/dL) or hemoglobin (up to 500 mg/dL).

3. Others

1) Use JP heparin as the calibration material. Caution must be exercised, because results will not necessarily be the same due to variation between manufacturers and lots. In addition, take care to prepare the heparin standard solution accurately, because the dilution ratio of the heparin standard solution is high.
2) Precautions for assay range
If the heparin level in the test plasma is high (≥ 0.8 IU/mL), dilute the sample with Normal Plasma and perform re-measurement.

Dosage/Administration (Assay Procedure) * *

1. Equipment

Centrifuge (for separation of plasma)
Thermostat
Spectrophotometer
Stopwatch
Mixer
Semi-microcuvette or 10 × 10 mm cuvette
Various micropipettes
Various measuring pipettes
Measuring cylinder
Plastic test tube
Tissue paper without waste textiles (such as Kim Wipe).

2. Methods of preparing the reagents and samples

1) Substrate Solution: Before use, dissolve 1 vial of the Substrate in 20 mL of purified water. After preparation, the solution is stable when stored for 3 months at 2–10 °C.
2) Antithrombin III Solution: Before use, dissolve 1 vial of the Antithrombin III in 10 mL of purified water. After preparation, the solution is stable when stored for 1 month at 2–10 °C.
3) Factor Xa Solution: Before use, dissolve 1 vial of the Factor Xa in 10 mL of purified water. After preparation, the solution is stable when stored for 1 month at 2–10 °C.
4) Buffer Solution: Buffer Solution is supplied as a ready-to-use reagent. After opening, store the Buffer Solution at 2–10 °C, and use it as soon as possible.
5) Normal Plasma: Before use, dissolve 1 vial of the Normal Plasma in 1.0 mL of purified water. After preparation, the plasma is stable when stored for 1 week at 2–10°C or for 1 month at –20°C.
6) Stop Solution (prepared in-house): Before use, add purified water to 20 g (20 mL) of glacial acetic acid to make a volume of 40 mL.
7) Heparin standard solution (tertiary diluted heparin solution) (prepared in-house):
   (1) Primary diluted heparin solution (10 IU/mL)
   Add physiological saline to 1 mL of 1000 IU/mL heparin sodium solution to make a volume of 100 mL, and use this solution as the primary diluted heparin solution.
   (2) Secondary diluted heparin solution (0.2 IU/mL)
   Add exactly 4.9 mL of the Buffer Solution to 100 µL of the primary diluted heparin solution (10 IU/mL), and use this solution as the secondary diluted heparin solution.
   (3) Heparin standard solution (tertiary diluted heparin solution)
   Dilute the secondary diluted heparin solution as directed in the following table and mix to prepare serial dilutions of the heparin standard solution.

Standard solution		Buffer Solution (µL)	Anti-thrombin III Solution (µL)	Normal Plasma (µL)	Secondary diluted heparin solution (µL)
No.	Heparin concentration (IU/mL plasma)				
1	0	800	100	100	0
2	0.2	700	100	100	100
3	0.4	600	100	100	200
4	0.6	500	100	100	300
5	0.8	400	100	100	400

8) Sample
   (1) Collect approximately 5 mL of citrated blood (whole blood: 0.1 mol/L sodium citrate = 9 volumes: 1 volume) from the patient by the dual syringe method.
   (2) Centrifuge the collected blood at 2000*g* for 20 minutes at 4°C, and immediately transfer the supernatant to a plastic tube for use as the test plasma.
   (3) Add 100 µL of the Antithrombin III Solution and 800 µL of the Buffer Solution to 100 µL of the test plasma, mix without bubbling, and use this solution as the sample. (Start the next operation as soon as possible.)

3. Assay Procedure

1) Endpoint method
   (1) Measure exactly 200 µL each of the sample for measurement and the blank sample into separate test tubes, and warm for 2–6 minutes at 37°C.
   (2) Add 100 µL of the Factor Xa Solution to the test tube for measurement, mix thoroughly, and warm for exactly 30 seconds at 37°C.
   (3) Add 200 µL of the Substrate Solution (previously warmed at 37°C) to the test tube for measurement, mix thoroughly, and warm for exactly 180 seconds at 37°C.
   (4) Add 300 µL of the Stop Solution to each test tube, and mix immediately.
   (5) Add 300 µL of purified water to the blank test tube, and mix.
   (6) Determine the absorbance of this solution at 405 nm using the blank as the control.
   (7) Proceed with each heparin standard solution as described for the sample for measurement, and determine the absorbance.

	Sample	Standard solution	Blank
Specimen	(Sample) 200 µL	(Heparin standard solution) 200 µL	(Sample or heparin standard solution) 200 µL
Warm for 2–6 minutes at 37°C.			
Factor Xa Solution	100 µL	100 µL	—
Mix, and warm for exactly 30 seconds at 37°C.			
(Previously warmed to 37°C) Substrate Solution	200 µL	200 µL	—
Mix, and warm for exactly 180 seconds at 37°C.			
Stop Solution	300 µL	300 µL	300 µL
Mix immediately.			
Purified water	—	—	300 µL
Determine the absorbance at 405 nm after mixing			

2) Method of measuring the initial rate

(1) Add 200 µL of the sample to the test tube, and warm for 2–6 minutes at 37°C.

(2) Add 100 µL of the Factor Xa Solution to the test tube, mix, and warm for exactly 30 seconds at 37°C.

(3) Add 200 µL of the Substrate Solution (previously warmed to 37°C) to the test tube, mix, and immediately transfer to the semi-microcuvette equipped with a thermostat (37°C).

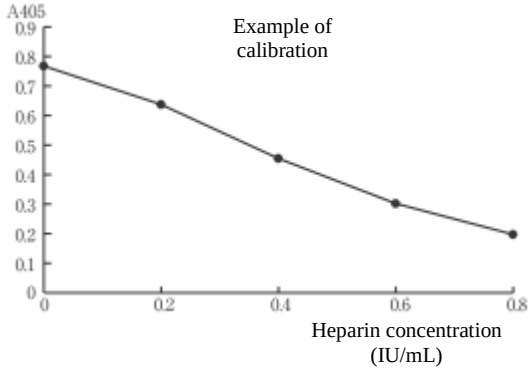
(4) Determine the change of absorbance at 405 nm per minute (ΔA/min) from 10 to 70 seconds after the start of the reaction.

(5) Proceed with each heparin standard solution as described for the sample, and determine the change of absorbance (ΔA/min).

	Sample	Standard solution
Specimen	(Sample) 200 µL	(Heparin standard solution) 200 µL
Warm for 2–6 minutes at 37°C.		
Factor Xa Solution	100µL	100µL
Mix, and warm for exactly 30 seconds at 37°C.		
(Previously warmed to 37°C) Substrate Solution	200µL	200µL
Immediately after mixing, transfer the mixture to a semi-microcuvette equipped with a thermostat (37°C), and determine the change of absorbance at 405 nm per minute (ΔA/min) from 10 to 70 seconds after the start of the reaction.		

3) Method of calculation

On a graph with the absorbance (or change of absorbance) on the y-axis and the heparin concentration on the x-axis, plot the absorbance (or change of absorbance) corresponding to the heparin concentrations in the serial dilutions of the heparin standard solution, and draw a line passing through each point to create the calibration curve. Use this calibration curve to determine the heparin concentration from the absorbance (or change of absorbance) of the sample.



Example of calibration

4) Precautions for testing

(1) Cool the collected blood on ice and prepare the test plasma as soon as possible, preferably within 90 minutes of blood collection. Measurement may be interfered if platelets are destroyed or there are too many contaminating platelets.

(2) Avoid vigorous mixing when preparing the Antithrombin III Solution, Factor Xa Solution, and Normal Plasma, because it may lead to deterioration of enzymes and degeneration of proteins.

(3) Warm the Factor Xa Solution (previously stored in a refrigerator) to room temperature before use.

(4) The components of the Substrate Solution may separate during storage in a refrigerator. If separation occurs, warm the solution at 50°C and use it after dissolution.

(5) If a standard 10 x 10 mm cuvette is used in place of the semi-microcuvette for the endpoint method, 1900 µL of 10% acetic acid may be added instead of 300 µL of the Stop Solution to stop the reaction. In this case, plot the calibration curve by adding 1900 µL of 10% acetic acid.

(6) After the reaction is stopped, the color remains stable for 4 hours.

Assessment of Assay Results

1. Reference treatment concentration[2)]

0.2–1.2 IU/mL

2. There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Performance

1. Sensitivity

1) Slope of the calibration curve: -0.78 to -0.42

2) Intercept of the calibration curve: 0.58 to 1.00

2. Accuracy: 90–110 % of the expected assay value

3. Within-run Reproducibility:

Coefficient of variation ≤ 5 %

(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[3)]: (Manual method)

0.15–0.8 IU /mL

5. Correlation[3)]

Plasma N=50 r=0.996 y=0.986x+0.00

Control method: Synthetic chromogenic substrate method

6. Standard Material

Heparin reference standard (NIBSC)

Precautions for Use or Handling ＊＊

1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.

2) Antithrombin III and Normal Plasma contain human-derived components that have been shown to be negative for HBs antigens, HIV antibodies, and HCV antibodies. However, these reagents (as well as the samples) should be considered potentially infectious and handled with great care.

2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the

reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.

2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life **

1. Storage temperature: 2–10°C

2. Shelf life: 2 years months from the date of manufacture

(The expiration date is printed on the outer package.)

Packaging

Name		Package
Testzym HeparinS	Substrate	1 × for 20 mL
	Antithrombin III	1 × for 10 mL
	Factor Xa	1 × for 10 mL
	Buffer Solution	1 × 100mL
	Normal Plasma	4 × for 1 mL

References *

1) Teien A.N. et al.: Thromb Res, 11, 107, 1977.
2) Hasegawa H. et al.: Jap Heart J, 21, 367, 1980.
3) In house data, SEKISUI MEDICAL CO., LTD.

Contact *

SEKISUI MEDICAL CO., LTD.
international@sekisui.com

Manufacturer **

SEKISUI MEDICAL CO., LTD.
1-3, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan

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