Snibe Diagnostic

CE 0123 REF 130218004M:100 tests/kit
130618004M: 50 tests/kit
130718004M: 30 tests/kit

MAGLUMI® Tissue plasminogen activator-Plasminogen activator inhibitor Complex (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Tissue plasminogen activator-Plasminogen activator inhibitor Complex (tPAIC) in human plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used to reflect the state of the fibrinolytic system and aid in the diagnosis of individuals with suspected or confirmed cardiovascular diseases.

SUMMARY

Tissue plasminogen activator-Plasminogen activator inhibitor Complex (tPAIC) is composed of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) complex with a molecular weight of about 110 kDa formed in a ratio of 1:1[1,2]. Among them, tPA is a serine protease with a molecular weight of about 64 kDa, which can activate plasminogen to plasmin and promote the degradation of fibrin clots[3]. PAI-1 is a serine protease inhibitor with a molecular weight of approximately 48 kDa, and the main physiological inhibitor of endogenous plasminogen activator (PA). It has the functions of inhibiting fibrin degradation, promoting fibrin deposition on the blood vessel wall, regulating intimal hyperplasia, etc, and is the main regulator of the fibrinolytic system[4,5]. The circulating concentration of tPA in plasma is very low, mostly in the form of a complex formed with PAI-1, and its half-life in plasma is very short. Therefore, the tPAIC content in plasma can be measured to reflect the increase of tPA in plasma [6,7]. Among them, increasing concentrations of thrombin produced a saturable, dose-dependent increase in the rate of release of tPA [8]. Plasminogen activation in plasma takes place only in the presence of fibrin, and occurs immediately when a thrombus is formed [9]. Fibrinolysis is activated by the release of tPA from damaged endothelium [10]. Hyperfibrinolysis diagnosed is associated with increased levels of tPA[11]. Therefore, the tPAIC level in the blood is a molecular marker that comprehensively reflects the activity of the fibrinolytic system, vascular endothelial cell damage and the presence of thrombus.

Monitoring of tPAIC levels is helpful for the diagnosis of early diffuse intravascular coagulation (DIC)[12], and auxiliary diagnosis of arteriovenous thrombosis caused by myocardial infarction[13], vasculitis[14], stroke[15], sepsis[16] and other basic diseases. Clinically, it is mainly used to reflect the state of the fibrinolytic system.

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with anti-PAI-1 monoclonal antibody and ABEI labeled with anti-tPA monoclonal antibody are mixed thoroughly and incubated, reacting to form sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of tPAIC present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-PAI-1 monoclonal antibody (~10.0 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of tPAIC antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of tPAIC antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	PBS buffer, NaN_3 (<0.1%).	6.5 mL	4.0 mL	3.0 mL
ABEI Label	ABEI labeled with anti-tPA monoclonal antibody (~83.3 ng/mL) in PBS buffer, NaN_3 (<0.1%).	12.5 mL	7.0 mL	4.8 mL
Diluent	PBS buffer, NaN_3 (<0.1%).	5.5 mL	3.5 mL	3.5 mL
Control 1	A low concentration of tPAIC antigen (5.00 ng/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of tPAIC antigen (20.0 ng/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
All reagents are provided ready-to-use.				

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Plasma	Sodium citrate (1:9)

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Samples must be free of fibrin and other particulate matter.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- The sample volume required for a single determination of this assay is 40 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 4 hours at 10-30°C or 24 hours at 2-8°C, or 12 months frozen at -20°C. Frozen specimens subjected to up to 1 freeze/thaw cycle have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, with tPAIC concentrations above the analytical measuring interval, can be diluted with Diluent either by following automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:10. The concentration of the diluted sample must be >10 ng/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

Tissue plasminogen activator-Plasminogen activator inhibitor Complex (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X3, MAGLUMI X6, MAGLUMI X8, MAGLUMI X8i, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the Snibe internal reference standard.

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 7 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other

published guidelines[17].
Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the tPAIC assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.
The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.
Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Tissue plasminogen activator-Plasminogen activator inhibitor Complex (CLIA) Controls (REF: 160201237MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the tPAIC concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/mL. For further information please refer to the Analyzer Operating Instructions.

Interpretation of Results

The expected range for the tPAIC assay was obtained by testing 571 apparently healthy individuals in China, gave the following expected value:

Apparently healthy population	N	95th percentile (ng/mL)
Female	285	≤10.5
Male	286	≤17.0

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory establish its own reference interval.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the tPAIC results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[18,19]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human plasma can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[20].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (ng/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Plasma Pool 1	10.276	0.273	2.66	0.153	1.49	0.397	3.86
Plasma Pool 2	39.638	1.015	2.56	0.368	0.93	1.252	3.16
Plasma Pool 3	79.837	1.548	1.94	1.273	1.59	2.637	3.30
Control 1	5.013	0.146	2.91	0.052	1.04	0.265	5.29
Control 2	19.915	0.551	2.77	0.209	1.05	0.656	3.29

Linear Range

1.00-100 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.700-1000 ng/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.400 ng/mL.
Limit of Detection (LoD) =0.700 ng/mL.
Limit of Quantitation (LoQ) =1.00 ng/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Intralipid	1000 mg/dL
HAMA	40 ng/mL
Rheumatoid factor	1500 IU/mL
ANA	398 AU/mL

High-Dose Hook

No high-dose hook effect was seen for tPAIC concentrations up to 1600 ng/mL.

Method Comparison

A comparison of the tPAIC assay with a commercially available immunoassay, gave the following correlations (ng/mL):
Number of samples measured: 235
Passing-Bablok: y=1.0096x-0.0491, τ=0.978.
The clinical specimen concentrations were between 1.056 and 99.057 ng/mL.

REFERENCES

1. Matsumoto H, Takeba J, Umakoshi K, et al. Successful treatment for disseminated intravascular coagulation (DIC) corresponding to phenotype changes in a heat stroke patient[J]. Journal of Intensive Care, 2019, 7(1).
2. Tjärnlund-Wolf A, Brogren H, Lo E H, et al. Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases[J]. Stroke, 2012, 43(10): 2833-2839.
3. Amiri M, Jalali-Javaran M, Haddad R, et al. In silico and in vivo analyses of the mutated human tissue plasminogen activator (mtPA) and the antithetical effects of P19 silencing suppressor on its expression in two Nicotiana species[J]. Scientific reports, 2018, 8(1): 1-13.
4. Iba T, Thachil J. Clinical significance of measuring plasminogen activator inhibitor-1 in sepsis[J]. Journal of Intensive Care, 2017, 5(1): 1-3.
5. Garg N, Goyal N, Strawn T L, et al. Plasminogen activator inhibitor-1 and vitronectin expression level and stoichiometry regulate vascular smooth muscle cell migration through physiological collagen matrices[J]. Journal of Thrombosis and Haemostasis, 2010, 8(8): 1847-1854.
6. Trauma induced coagulopathy[M]. Cham, Switzerland: Springer International Publishing, 2016:34.
7. Urano T, Castellino F J, Suzuki Y. Regulation of plasminogen activation on cell surfaces and fibrin[J]. Journal of Thrombosis and Haemostasis, 2018, 16(8): 1487-1497.
8. Levin E G, Marzec U, Anderson J, et al. Thrombin stimulates tissue plasminogen activator release from cultured human endothelial cells[J]. The Journal of clinical investigation, 1984, 74(6): 1988-1995.
9. Urano T, Suzuki Y, Iwaki T, et al. Recognition of plasminogen activator inhibitor type 1 as the primary regulator of fibrinolysis[J]. Current drug targets, 2019, 20(16): 1695-1701.
10. Harvey J W, Stevens A, Lowe J S, et al. Veterinary hematology[M]. St. Louis: WB Saunders, 2012:202.
11. Moore H B, Moore E E, Liras I N, et al. Acute fibrinolysis shutdown after injury occurs frequently and increases mortality: a multicenter evaluation of 2,540 severely injured patients[J]. Journal of the American College of Surgeons, 2016, 222(4): 347-355.
12. Heng, Mei, Ying, et al. Evaluation the combined diagnostic value of TAT, PIC, tPAIC, and sTM in disseminated intravascular coagulation: A multi-center prospective observational study.[J]. Thrombosis research, 2018, 173:20-26.
13. Björn Wiman, Andersson T , Hallqvist J, et al. Plasma Levels of Tissue Plasminogen Activator/Plasminogen Activator Inhibitor-1 Complex and von Willebrand Factor Are Significant Risk Markers for Recurrent Myocardial Infarction in the Stockholm Heart Epidemiology Program (SHEEP) Study[J]. Arteriosclerosis Thrombosis & Vascular Biology, 2000, 20(8):2019.
14. Senzaki H, Kobayashi T, Nagasaka H, et al. Plasminogen Activator Inhibitor-1 in Patients with Kawasaki Disease: Diagnostic Value for the Prediction of Coronary Artery Lesion and Implication for a New Mode of Therapy[J]. Pediatric Research, 2003, 53(6):983.
15. Johansson L, Jansson J H, Boman K, et al. Tissue plasminogen activator, plasminogen activator inhibitor-1, and tissue plasminogen activator/plasminogen activator inhibitor-1 complex as risk factors for the development of a first stroke[J]. Stroke, 2000, 31(1): 26-32.
16. Mavrommatis A C, Theodoridis T, Economou M, et al. Activation of the fibrinolytic system and utilization of the coagulation inhibitors in sepsis: comparison with severe sepsis and septic shock[J]. Intensive care medicine, 2001, 27(12): 1853-1859.
17. CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
18. Primus F J, Kelley E A, Hansen H J, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy[J]. Clinical Chemistry, 1988, 34(2):261-264.
19. Robert W. Schroff, Kenneth A. Foon, Shannon M. Beatty, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy.[J]. Cancer Research, 1985, 45(2):879-885.
20. Boscato L M, Stuart M C. Heterophilic antibodies: a problem for all immunoassays[J]. Clinical Chemistry, 1988, 34(1):27-33.

SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE 0123	CE marking with notified body ID number		

Summary of safety and performance is available at Eudamed.

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax:+86-755-28292740

EC REP **Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726