# Instructions for Use

# Thrombin-Anti-thrombin III Complex (CLIA)

**【Product Name】**

Thrombin-Anti-thrombin III Complex (CLIA)

**【Packing Size】**

60×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg

**【Intended Use】**

This product is intended for use in quantitative determination of thrombin-anti-thrombin III complex (TAT) in human plasma samples. It is mainly used for auxiliary diagnosis of thrombotic diseases in the clinics.

Thrombin-anti-thrombin III (TAT) is a covalently linked complex formed by rapid binding of anti-thrombin (AT) to thrombin (T) generated in vivo. The formation of TAT reflects the generation of thrombin and marks the activation of the coagulation cascade. Quantitative measurement of TAT concentration in plasma is of great significance for the diagnosis of thrombosis such as disseminated intravascular coagulation (DIC).

**【Principle】**

This assay adopts a double-antibody sandwich chemiluminescence immunoassay format. The test principle is as follows:

(1) Add sample and magnetic microparticle coated with thrombin (T) antibody to the reaction well. After mixing and incubation, TAT in the sample binds to the T antibody coated on the magnetic microparticle. After the reaction is completed, a magnet is used to attract the magnetic microparticle, and the washing solution is added to wash away unbound substances.

(2) Then, the acridinium-labeled anti-thrombin III antibody (AT) is added. After mixing and incubation, the acridinium-labeled AT antibody will bind to TAT on magnetic microparticle to form antibody (T)-antigen (TAT)-antibody (AT) sandwich complex.

(3) After the reaction is complete, a magnet is used to capture the microparticle, unbound material is washed away. Then, add pre-trigger solution and trigger solution into the reaction mixture sequentially to initiate chemiluminescent reaction.

(4) A photomultiplier tube is used to measure photons generated from the reaction. The count of photons is in direct proportion to TAT concentration in the sample. TAT concentration is derived from a built-in calibration curve.

**【Main Components】**

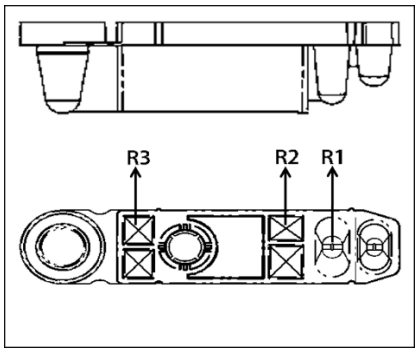
| Component | Main Composition | Fill Volume | |
|---|---|---|---|
| | | 60 x 1 Tests/Pkg (Calibrator included) | 60 x 1 Tests/Pkg |
| TAT reagent cartridge | **Microparticle (R1):**Magnetic microparticle coated with mouse anti-Thrombin monoclonal antibody, ~0.2g/L; HEPES buffer, 50mmol/L; ProClin 300, 0.5g/L | 60×50μL | 60×50μL |
| | **Conjugate (R2):**Mouse anti-AT monoclonal antibody labeled with acridinium, ~200μg/L; MES buffer, 50mmol/L; ProClin 300, 0.5g/L | 60×100μL | 60×100μL |
| | **Reaction diluent (R3):**HEPES buffer, 50mmol/L; ProClin 300, 0.5g/L | 60×100μL | 60×100μL |
| TAT calibrator C1 | TAT antigen; HEPES buffer, 50mmol/L; ProClin 300, 0.5g/L | 1×1.0mL | / |
| TAT calibrator C2 | TAT antigen; HEPES buffer, 50mmol/L; ProClin 300, 0.5g/L | 1×1.0mL | / |
| Reconstitution solvent | Deionized water | 2×1.0mL | / |
| Calibration card | A card containing information of calibrator lot number and calibrator concentration | 1 pcs | / |

Note: Components in different lots of reagent cannot be exchanged for use.

Traceability: This testing method is traceable to the measurement procedure of Sysmex system selected by the enterprise.

After scanning the calibration card, relevant calibrator information (calibrator lot No. and concentration) is checked into the instrument.

The position of each component is shown in the front view (Upper) and vertical view (Down) of the reagent pack.




**Instrument or material required but not provided (available from Medcaptain):**

(1) Immu F6/ F6S Automated Chemiluminescence Immunoassay Analyzers;
(2) Washing buffer;
(3) Pre-trigger solution;
(4) Trigger solution;
(5) Sample diluent;
(6) 500μL pipette tips;
(7) TAT Controls

**(8) 【Storage Condition and Shelf Life】**

Storage condition: Sealed for storage at 2~8 ℃ in an upright position. Avoid freeze-thaw cycle.

Shelf life: 14 months.

Calibrator stability: If the calibrator is capped and stored at 2~8 ℃, the shelf life of the calibrator is 14 months. After reconstitution, the calibrator is allowed to be stored for 8 hours at room temperature (10~30 ℃), for 3 days at 2~8 ℃, and for 30 days at -20 ℃ or below. Freeze-thaw once only.

Manufacturing and expiration dates can be found on labels and outer package.

**【Applicable Instrument】**

Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzers

**【Sample Preparation】**

- Sodium citrate plasma is used for the test.
- Sample volume for each test: 50 μL
- The collected sample should be tested as soon as possible.
- Plasma samples are allowed to be stored for up to 8 hours at room temperature (10~30 ℃), 24 hours at 2~8 ℃, and 30 days at -20 ℃ or below. A sample cannot be frozen and thawed multiple times. Only one freeze-thaw cycle is allowed. If a sample contains sediment or floccule after freeze and thaw, centrifuge the sample before testing.
- Blood collection tubes provided by different manufacturers are different in raw materials and additives, which may impact the test results. Medcaptain has not validated all types of blood collection tubes from different manufacturers that might be used for this assay. Each laboratory must make its own judgment about the feasibility of using certain brands of blood collection tubes.

**【Test Procedure】**

**Reagent preparation**

Reagent: TAT reagent cartridges (including microparticle R1, conjugate R2, and reaction diluent R3) is ready for use. The cartridge can be loaded to the instrument directly.

Calibrators: TAT calibrators C1 and C2 are lyophilized powders and need to be reconstituted before use. Pour a vial of reconstitution solvent (1.0mL/vial) in the kit into a vial of TAT calibrator C1 or C2; Seal it with a stopper, invert it several times and avoid bubbles. Let it stand for about 10 minutes until the calibrator is completely reconstituted. After reconstitution, the calibrator can be split into aliquots, packaged, labeled, and stored under proper conditions for future use (see 【Storage Condition and Shelf Life】). The calibrator aliquot can be used only once.

**Calibration**

- Refer to the operation manual of Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzer for system calibration.
- Calibration must be performed at least once when a new lot of reagent is to be used.
- Use TAT assay kit and matched calibrators provided by Medcaptain for analyzer calibration.
- Before calibration, scan the calibration card provided in TAT kit to input the calibration curve and calibrator information.
- When performing calibration, take out reagent cartridges from the package, place it on reagent cartridge holder, push it in and close the door. Reagent-related information (reagent name, lot No., and expiration date) can be read automatically through a two-dimensional barcode on each reagent cartridge.
- Put calibrators on a sample rack, and push the sample rack into the sampling area on the analyzer.
- Select **Reagent** > **Request Calib.** on the screen interface of the analyzer, and select the corresponding assay and lot No. to request a calibration.
- Select the positions of calibrators on the sample rack, set the repeated number of tests, and start calibration.
- Based on calibrator test results, the analyzer system automatically checks the validity of the calibration curve, and makes adjustment to generate a calibration curve.
- Validity period of calibration: 28 days
- Renewed calibration is required in the following cases:

(1) Before using assay kits of a different lot;

(2) When the control test results exceed the specified limits;

(3) When the same lot of reagent has been used on the instrument for over 28 days.

- For details about calibration, refer to the section about calibration in the operation manual of Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzer.

**Control test**

- There are two levels of TAT Controls: low-concentration TAT control (L) and high-concentration TAT control (H).
- These two TAT controls should be tested at least once every 24 hours when the TAT assay is in use. Control testing is highly recommended every time the lot of reagent has been changed, the instrument needs to be re-calibrated, or after trouble shooting/ maintenance services.
- When performing the control test, take out the reagent cartridges from the package, place them on reagent cartridge holder and close the door. Reagent-related information (reagent name, lot No., and expiry date) can be read automatically via a two-dimensional barcode on each reagent cartridge.
- Put the controls on a sample rack, and push the sample rack into the sampling area on the analyzer.
- On the test request interface, choose “Control” as the test type and select the control lot No. and assay name for the control test.
- Press the start button to start the test. The control test results can be viewed after the test is finished.
- The control test results should fall within the defined limits. Otherwise, check the test system to identify the root cause, i.e., check the expiration date and storage condition of the controls, the analyzer performance and status. After eliminating the problems, test the controls again. If the test result still exceeds the range, please contact Medcaptain customer service immediately.
- Each laboratory is recommended to establish its own control intervals and limits, based on its own conditions.
- For details about the control test, refer to the section about control test in the operation manual of Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzer.

**Sample Test**

- When performing the sample test, take out reagent cartridges, place them on the reagent cartridge holder, and close the door. Reagent-related information (reagent name, lot No., and expiration date) can be read automatically via a two-dimensional barcode on the reagent cartridge.

- If a blood collection tube is used in the test, sample volume must be larger than 1.0mL.
- Uncap the sample tubes, place samples on the sample rack, and push the sample rack into the sampling area on the instrument.
- On the test request interface, choose "Sample" as the test type, enter sample information, and select TAT assay for the test.
- Press the start button to run the test. Test results can be viewed after the test is completed.
- Required volume of each reagent component, 50μL of R1, 100μL of R2, and 100μL of R3 for one test. The analyzer automatically pipettes and mixes the sample, R1 and R3, and incubate the mixture at 37℃. After the incubation, the immune complex is washed, and then 100μL of R2 is pipetted and mixed, and incubated at 37℃ for 5 minutes. After the incubation, the immune complex is washed again, and the pre-trigger solution is added to incubate with the immune complex for 1 min, and then the trigger solution is added to initiate chemluminescence signal. The time from sample pipetting to test completion is about 20 min.
- For details about sample test, refer to the section about the sample test in the operation manual of Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzers.

**Calculation**

Based on the lot-specific calibration curve, the instrument automatically calculates the test results of each sample (unit: ng/mL).

**【Reference Intervals】**

The 95th percentile reference interval is <4.08 ng/mL through the study of 259 healthy normal people (130 males and 129 females, aged from 18 to 87) in Guangdong,. Due to differences in geographic region, race, gender, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each laboratory to establish its own reference intervals.

**【Interpretation of Results】**

- The test results obtained by using this assay kit can only be used for clinical reference. It cannot be used as the only basis for confirming or ruling out a disease. Clinical symptoms, medical history, other laboratory test results, and therapeutic response must be combined for confirming the diagnosis or ruling out the possibility of a disease in a comprehensive manner.
- The measurement range of this assay is 0.4~120.0ng/mL. If the TAT concentration in a sample is lower than the limit of detection (LoD), the value will be reported as "<0.4ng/mL". If the TAT concentration in a sample is higher than the upper limit of measurement, the value will be reported as ">120ng/mL".
- For a sample with TAT concentration over 120ng/mL, it is recommended to manually dilute the sample using sample diluent (the recommended dilution rate is 1:10), and perform the test again to obtain an accurate result.
- When the instrument displays "SMPL", the sample is insufficient. In this case, prepare sufficient sample for the test again.
- Some test results may contain identification symbols. For details about the identification symbols, refer to the section about result identification in the operation manual of Medcaptain Immu F6/F6S Automated Chemiluminescence Immunoassay Analyzer.

**【Limitation】**

- The test results obtained by using this assay kit can only be used for clinical reference. It cannot be used as the only basis for confirming or ruling out a disease.
- No Hook effect occurs for TAT concentration lower than 2000ng/mL.
- When concentration of endogenous interfering substance is lower than the value listed in the table below, relative deviation of the measurement value caused by interference will not exceed ±10%.

| Potential Endogenous Interference Substances | Concentration of Potential Interference Substances |
|---|---|
| Total protein | ⩽12g/dL |
| Bilirubin | ⩽21mg/dL |
| Hemoglobin | ⩽500mg/dL |
| Triglyceride | ⩽1500mg/dL |

- When the potential cross-reactant is at the following concentration, the measured concentration will not exceed 0.4ng/mL.

| Potential Cross Reactant | Concentration |
|---|---|
| Prothrombin | ⩽150 μ g/mL |
| Anti-thrombin III | ⩽310 μ g/mL |

- Heterophilic antibody and rheumatoid factor (RF) in human blood can react with immunoglobulin in the reagent, which will impact assay results. Consequently, abnormal value may be observed in the test. For this reason, other clinical information must be combined with the test results to make a comprehensive diagnosis.

- Heterophilic antibodies may develop in patients who have been in frequent contact with animals, or have been treated/diagnosed with immunoglobulins or immunoglobulin fragments. For example, some patients who have received mouse monoclonal antibody treatment or diagnosis may contain human anti-mouse antibody (HAMA), and test results of these patients may be falsely elevated or reduced. This assay kit also contains ingredient which can diminish interference from HAMA and anti-antinuclear antibody (ANA). However, a few samples may still have the problem of HAMA and ANA interference. Additional clinical or diagnostic information is required to help physicians to make the final judgment on patients. .

- The clinical samples tested as ANA positive by antinuclear antibody IgG detection kit (indirect immunofluorescence method) were used for interference evaluation, which showed that the deviation of its influence on the test results was within ±10%.

- When the concentration of RF is lower than 200 IU/mL and the concentration of HAMA is lower than 1000 ng/mL, the deviation of the test results is within ±10%.

**【Product Characteristics】**

**1 Limit of Blank (LoB)**

LoB ⩽ 0.2ng/mL.

**2 Limit of Detection (LoD)**

LoD ⩽ 0.4ng/mL.

**3 Accuracy**

The accuracy must meet one of the following requirements:

a) Relative deviation: Test the traceable accuracy reference samples at two concentration levels, the relative deviation of the measurement results from the

target value should not exceed ±10.0%.

b) Spiked recovery test: Add a known concentration of TAT to real TAT samples, and the recovery rate should be within the range of 100.0±15.0 %.

**4 Linearity**

Test TAT samples with concentration in the range of 0.4~120ng/mL. Linear correlation coefficient r≥0.990.

**5 Repeatability**

Coefficient of variation (CV) for the test results of low (4.0±0.8)ng/mL and high (20.0±4.0)ng/mL corporate reference sample is: CV ≤8.0%.

**6 Batch-to-Batch Variation**

Coefficient of variation (CV) for the test results of low (4.0±0.8)ng/mL and high (20.0±4.0)ng/mL corporate reference sample with three batches of reagent is: CV ≤10.0%.

**7 Accuracy of Assigned Value**

Use the primary calibrators with values assigned using a higher-level measurement procedure to calibrate the chemiluminescence immunoassay system. Afterwards, use the same lot of assay kit to measure the value of each product calibrator. The deviation between measurement value and assigned value does not exceed ±10.0%.

**8 Homogeneity of Calibrators**

8.1 Within-vial Homogeneity

Coefficient of variation (CV) is used to evaluate the within-vial homogeneity of calibrators C1 and C2, which should be less than 8.0%.

8.2 Between-vial Homogeneity

Coefficient of variation (CV) is used to evaluate the between-vial homogeneity of calibrators C1 and C2, which should be less than 5.0%.

**【Precautions】**

1 This product is for IVD use only.

2 This product is intended to be used by professionals only.

3 Do not use the product beyond its expiration date.

4 Do not pool reagents from different reagent lots.

5 Due to the difference in antibody specificity, systems of different manufacturers may generate different results for the same sample. Test results obtained from different systems may not be comparable, and should not be correlated to each other in clinical interpretation.

6 Do not vehemently shake reagent components to avoid bubble formation.

7 During use of this product, perform tests by strictly following operation procedures as written in the package insert and guidelines set up by each laboratory.

8 The test results from this assay can only be used as auxiliary evidence for clinical decision. Clinical symptoms, medical history, other laboratory test results, and therapeutic response must be combined for patient management in a comprehensive manner.

9 Lab operator must wear suitable gloves and lab coat when using the product. In case of accidental exposure to the reagent, flush the body part with large volume of water immediately. If reagent splashes into eyes, flush eyes with copious of water and consult a doctor immediately.

10 All samples and reaction wastes must be considered potentially biohazards, and be handled in accordance with the local laws and regulations.

11 Do not re-use a reagent cartridge. It is designed for single use only.

12 Put left-over reagent cartridges back into 2~8℃ refrigerator, rather than leave them on the instrument for extended period of time.

**【Interpretation of Symbols】**

| | | | |
|---|---|---|---|
| | Temperature limit | | Date of manufacturing |
| IVD | *In vitro* diagnostic medical device | REF | Catalogue number |
| LOT | Batch Code | | Consult instruction for use or consult electronic manual for use |
| | Use-by Date | EC REP | Authorized representative in the European Community/ European Union |
| | This way up | CE | CE marking |
| | Manufacturer | UDI | Unique device identifier |

**【References】**


[1] Wada H. Hemostatic molecular markers before the onset of disseminated intravascular coagulation. Seminars in Thrombosis & Hemostasis, 1998, 24(03):293-297.

[2] Kawasaki T, Shinoki N, Iwamoto S, et al. Diagnostic value of plasma thrombin-antithrombin III complex and D-dimer concentration in patients with varicose Veins for exclusion of deep-vein thrombosis. Thrombosis Research, 1998, 91(2):101-104.

[3] Estivals M, Pelzer H, Sie P, et al. Prothrombin fragment 1+2, thrombin–antithrombin III complexes and D-dimers in acute deep vein thrombosis: effects of heparin treatment. British Journal of Haematology, 1991, 78(3):421-424.


**[Basic Information]**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