SIEMENS Healthineers

Enzygnost® TAT micro

CE 0197

Revision bar indicates update to previous version.

Intended Use

Enzygnost® TAT micro is an in vitro diagnostic reagent for the quantitative determination of thrombinantithrombin complex (TAT) as an aid to diagnosis and monitoring of hypercoagulable states in patients with suspicion or signs of blood coagulation disorders in human sodium citrated plasma by means of semiautomated ELISA technology.

For thrombin-antithrombin complex testing no international reference preparation or method is available.

Summary and Explanation

The conversion of prothrombin into active thrombin is a key event in the coagulation cascade. Active thrombin has a very short half and is inactivated by complex formation with antithrombin in less than a minute. The immunochemical determination of the resulting thrombin-antithrombin complex (TAT) allows the precise quantification of the thrombin generated in-vivo thrombin within the last 30 to 60 min[1].

TAT is a sensitive marker of in vivo thrombin generation, with elevated TAT levels indicating increased coagulation activation such as pro-thrombotic states and consumption coagulopathy.

Determination of TAT may aid in the assessment of the hemostatic balance in the following clinical situations:

- Marker for non-overt DIC[3,4]
- Monitoring of pregnant women with thrombophilia, preeclampsia or HELLP syndrome[5,6]
- Prognostic marker in patients with acute cardiac events in combination with cardiac markers[7,8]
- Marker of hypercoagulability and venous thromboembolism in cancer patients[9–11]

Principle of the Method

Enzygnost® TAT micro is a sandwich enzyme immunoassay for the *in-vitro* determination of human thrombin/antithrombin III complex.

During the first incubation step the TAT present in the sample binds to the antibodies against thrombin which are attached to the surface of the microtitration plate. Unbound constituents are then removed by washing and, in a second reaction, peroxidase-conjugated antibodies to human AT III are bound to the free AT III determinants. The excess enzyme-conjugated antibodies are removed by washing; the bound enzyme activity is then determined. The enzymatic reaction between hydrogen peroxide and chromogen is terminated by the addition of diluted sulphuric acid. The resulting colour intensity, which is proportional to the concentration of TAT, is determined photometrically. The concentration range of 2 to 60 µg/L is covered by the standards contained in the kit. For higher concentrations the sample must be diluted with normal plasma.

Reagents

Reagent	Description	Storage	Stability
Enzygnost® TAT micro			
MTP	Mictrotiter plate, coated: • antibodies to human thrombin, rabbit (< 192 µg/plate)	2–8 °C	2–8 °C[a]: once opened, 4 weeks
CONJUGATE	Ready to use liquid containing: • anti-human ATIII, rabbit (peroxidase-conjugated) (< 12 mg/L) • Preservative: • Phenol (< 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 4 weeks; ≤ –20 °C: once opened, 3 months
REAGENT DILUENT	Ready to use liquid containing: • TRIS (50 mmol/L) • Bovine serum albumin • Sodium azide (< 1 g/L) • Preservative: • Phenol (< 0.3 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 4 weeks; ≤ –20 °C: once opened, 3 months
STANDARD 1	Lyophilized reagent containing: • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: ~45 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours; ≤ –20 °C: reconstituted, 5 weeks
STANDARD 2	Lyophilized reagent containing: • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: ~45 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours; ≤ –20 °C: reconstituted, 5 weeks
STANDARD 3	Lyophilized reagent containing: • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: ~45 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours; ≤ –20 °C: reconstituted, 5 weeks
STANDARD 4	Lyophilized reagent containing: • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: ~45 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours; ≤ –20 °C: reconstituted, 5 weeks
CONTROL	Lyophilized reagent containing: • Preservative: • reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (reconstituted: ~45 mg/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C: reconstituted, 8 hours; ≤ –20 °C: reconstituted, 5 weeks

Reagent	Description	Storage	Stability
DILUENT	Ready to use liquid containing: • TRIS (100 mmol/L) • Tween 20 (~10 g/L) • EDTA (37 g/L) • Preservative: • Sodium azide (< 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, 4 weeks; ≤ –20 °C: once opened, 3 months
WASH POD	Ready to use liquid containing: • Phosphate buffer solution (90 mmol/L) • Tween 20 (18 g/L) • Preservative: • Phenol (≤ 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, n/a[b]; 2–8 °C: diluted 1:20, 1 week
SUBSTRATE POD	Ready to use liquid containing: • Hydrogen peroxide solution (~0.3 g/L) • Preservative: • n-butanol (< 1 %)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, n/a[b]
CHROMOGEN POD	Lyophilized reagent containing: • o-phenylenediamine dihydrochloride (reconstituted: 13.5 mmol/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	15–25 °C[c]: reconstituted, 1 hour
STOP POD	Ready to use liquid containing: • sulfuric acid (0.25 mol/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	2–8 °C: once opened, n/a[b]
Adhesive foils			

[a] in the bag with desiccant
[b] expiry date
[c] protected from light

Working conjugate solution (CONJUGATE diluted with REAGENT DILUENT) can be used within a maximum of 4 weeks stored at 2 to 8 °C.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.



Danger! STOP | POD
Hazardous ingredient: sulfuric acid (2.47 % [w/w]).
H290: May be corrosive to metals. **H314**: Causes severe skin burns and eye damage.
P234: Keep only in original container. **P264**: Wash hands thoroughly after handling. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P390**: Absorb spillage to prevent material damage. **P301 + P330 + P331**: IF SWALLOWED: rinse mouth. Do NOT induce vomiting. **P303 + P361 + P353**: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. **P305 + P351 + P338**: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310**: Immediately call a POISON CENTER or doctor/physician. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.



Danger! CHROMOGEN | POD
Hazardous ingredient: o-phenylenediamine dihydrochloride (71.3 % [w/w]).
H301: Toxic if swallowed. **H312 + H332**: Harmful in contact with skin or if inhaled. **H319**: Causes serious eye irritation. **H317**: May cause an allergic skin reaction. **H341**: Suspected of causing genetic defects. **H351**: Suspected of causing cancer. **H410**: Very toxic to aquatic life with long lasting effects.
P201: Obtain special instructions before use. **P261**: Avoid breathing dust. **P280**: Wear protective gloves/protective clothing/eye protection/face protection. **P264**: Wash hands thoroughly after handling. **P273**: Avoid release to the environment. **P301 + P310**: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. **P312**: Call a POISON CENTER or doctor/physician if you feel unwell. **P337 + P313**: If eye irritation persists: Get medical advice/attention. **P308 + P313**: IF exposed or concerned: Get medical advice/attention. **P302 + P352**: IF ON SKIN: Wash with plenty of soap and water. **P333 + P313**: If skin irritation or rash occurs: Get medical advice/attention. **P362 + P364**: Take off contaminated clothing and wash it before reuse. **P391**: Collect spillage. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.



Warning! STANDARD 1, STANDARD 2, STANDARD 3, STANDARD 4, CONTROL
Hazardous ingredient: reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one / 2-methyl-2H-isothiazol-3-one (3:1) (0.0778 % [w/w]).
H315: Causes skin irritation. **H317**: May cause an allergic skin reaction. **H319**: Causes serious eye irritation. **H411**: Toxic to aquatic life with long lasting effects.
P280: Wear protective gloves/protective clothing/eye protection/face protection. **P273**: Avoid release to the environment. **P261**: Avoid breathing dust. **P264**: Wash hands thoroughly after handling. **P391**: Collect spillage. **P302 + P352**: IF ON SKIN: Wash with plenty of soap and water. **P333 + P313**: If skin irritation or rash occurs: Get medical advice/attention. **P362 + P364**: Take off contaminated clothing and wash it before reuse. **P337 + P313**: If eye irritation persists: Get medical advice/attention. **P501**: Dispose of contents and container in accordance with all local, regional, and national regulations.



CAUTION! POTENTIAL BIOHAZARD
STANDARD 1, STANDARD 2, STANDARD 3, STANDARD 4, CONTROL
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests that are CE marked or FDA approved for this purpose. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Caution
CONJUGATE, REAGENT DILUENT, STANDARD 1, STANDARD 2, STANDARD 3, STANDARD 4, CONTROL, DILUENT
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Preparing Reagents

Satisfactory performance of the test is assured only if the reagents used have the required lot no.; the required lot nos. are listed on the pack.

WASH POD: Dilute 15 mL to 300 mL with distilled or demineralized water (sufficient for 2 test plates). If fewer than 2 test plates are required, a corresponding portion of the WASH POD is to be diluted with water.

Working Conjugate Solution: Pipette 200 µL of CONJUGATE into a vial of REAGENT DILUENT (11 mL) and shake gently to mix (sufficient for 1 test plate).

STANDARD 1 to STANDARD 4 and CONTROL (human): Reconstitute each in 1 mL of distilled water or demineralized water. STANDARD 1 to STANDARD 4 then contain 2, 6, 20 and 60 µg/L TAT respectively and the control contains the value given on the label (e.g. 10 ±2 µg/L).

Bring all the reagents and samples to 15 to 25 °C before the start of the test.

SUBSTRATE POD, **Working Chromogen Solution** and STOP POD must not be allowed to contact heavy metal ions or oxidizing substances (do not use pipettes with metal parts). If laboratory glassware is employed to prepare comparatively large amounts of the Working Chromogen Solution, it should be rinsed well beforehand and dried at 120 °C.

Specimen Collection and Handling

Collecting the Specimen

To obtain the plasma, carefully mix 1 part citrate buffer solution or sodium citrate solution 0.11 mol/L (3.2 %) with 9 parts venous blood, avoiding the formation of foam. Centrifuge immediately at no less than 1 500 × g for at least 15 minutes and remove the supernatant plasma.

Storing the Specimen

Stability of the samples:

2 to 8 °C	4 hours
15 to 25 °C	4 hours
≤ –20 °C	1 month

Procedure

Materials Provided

REF	Contents		Number of Tests
OWMG15	Enzygnost® TAT micro		2 × 96
	Enzygnost® TAT micro microtitration plate MTP	2 pcs.	
	Anti-ATIII/POD Conjugate CONJUGATE	1 × 0.5 mL	
	Conjugate Buffer (coag.) REAGENT DILUENT	2 × 11 mL	
	TAT Standard Plasma S1 STANDARD 1	1 × → 1 mL	
	TAT Standard Plasma S2 STANDARD 2	1 × → 1 mL	
	TAT Standard Plasma S3 STANDARD 3	1 × → 1 mL	
	TAT Standard Plasma S4 STANDARD 4 Table of Analytical Values	1 × → 1 mL	
	TAT Control Plasma CONTROL Table of Assigned Values	1 × → 1 mL	
	Sample Buffer (TAT) DILUENT	1 × 20 mL	
	Washing Solution POD (concentrate) WASH POD	1 × 100 mL	
	Buffer/Substrate POD SUBSTRATE POD	1 × 50 mL	
	Chromogen POD CHROMOGEN POD	4 × → 10 mL	
	Stopping Solution POD STOP POD	1 × 100 mL	
	Adhesive foils	6 pcs.	

Materials Required but not Provided

Item	Description
–	Piston-type pipettes: 50 µL, 100 µL, 200 µL For performing the test manually: Incubation: Water bath (37 °C, circulating) Washing equipment: Microtiter plate washer or a dispenser for about 0.3 mL with an aspiration system Photometer: Photometer suitable for reading microtiter plates, measuring wave length 492 nm (490 to 500 nm)

Please note that the applications on automatic analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Test Procedure

Note: All the determinations (standards, control, patient samples) are to be performed in duplicate. The reference curve must be established for each series of measurements using the standards. In order to assure the accuracy and precision of Enzygnost® TAT micro, the samples should be pipetted within 5 minutes, e.g. by using a sample processor station or with the aid of a multi-channel pipette and the Micronix-System. Alternatively, use a second standard series after 5 minutes for the remainder of the microtiter plate. **The incubation period begins when the plate is placed in the water bath!**

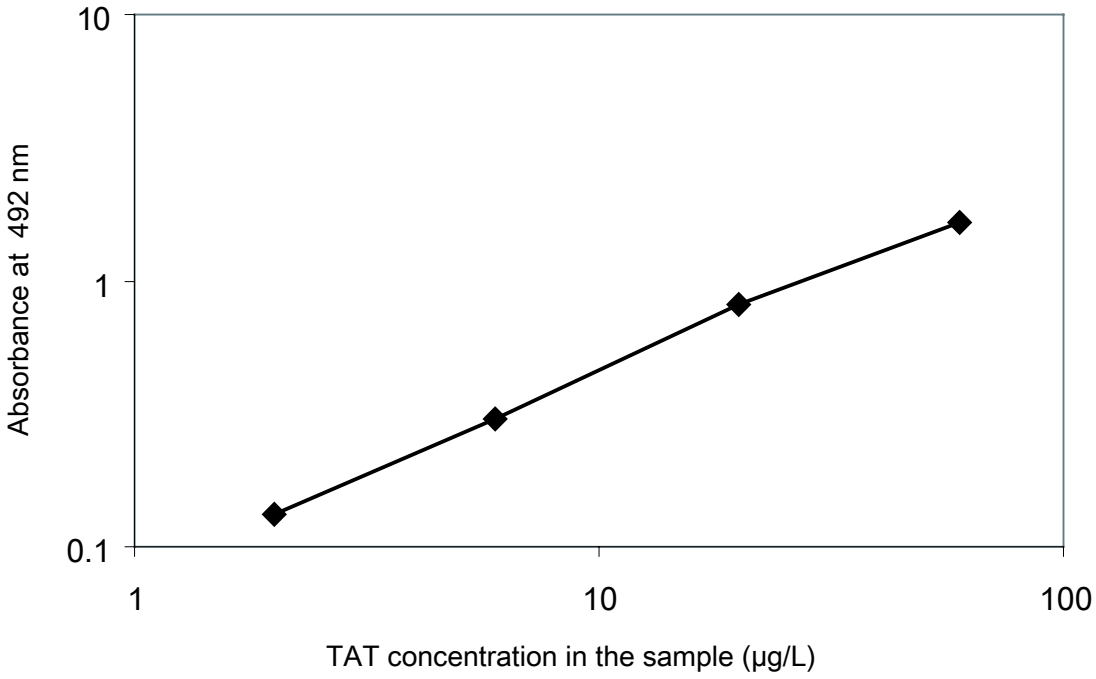
During the pipetting steps care should be taken to avoid the formation of comparatively large bubbles. The incubation times can be freely selected from within the given ranges but must be the same for all the assay mixtures within a series.

1. Ascertain the number of strips required (each strip contains 8 wells, 2 wells each are required for standards, control, and sample). Remove from the holder strips which are not required and store well-closed with the desiccant in the plastic bag at 2 to 8 °C.
2. Into each well pipette a receiving volume of 50 µL Sample Buffer (TAT). To each well then add 50 µL standard, control or sample. After filling the test plates shake briefly to ensure thorough mixing.
3. Cover with self-adhesive foil and incubate for 15 minutes (±2 minutes) at 37 °C.
4. Remove the self-adhesive foil, aspirate all the wells, fill with approx. 0.3 mL diluted washing solution and aspirate again. Repeat this washing step two more times, then remove any remaining washing solution by knocking the test plate on a cellulose tissue.
5. Pipette 100 µL of Working Conjugate Solution into each well (starting at tube 1), taking care not to wet the edges of the wells if possible.
6. Cover with fresh self-adhesive foil and incubate for 15 minutes (±2 minutes) at 37 °C.
7. Prepare the **Working Chromogen Solution**: shortly before the end of the incubation period, transfer 10 mL Buffer/Substrate POD (temperature 15 to 25 °C) into a vial of Chromogen POD and shake to dissolve.
8. Remove the foil, aspirate all the wells and wash three times as described above, then remove any remaining liquid by knocking the test plate on a cellulose tissue.
9. To each well add 100 µL of the freshly prepared Working Chromogen Solution.
10. Cover with fresh foil, incubate for 30 minutes (±2 minutes) at 15 to 25 °C **protected from light** (e.g. in a dark drawer).
11. Remove the foil and add 100 µL of Stopping Solution POD, keeping to the same timing as for the dispensing of the Working Chromogen Solution (Step 9).
12. Measure with a spectrophotometer within one hour; measuring wavelength 492 nm (490 to 500 nm).

Evaluation

After calculating the mean absorbance values of the standards plot the reference curve on log-log graph paper.

Performing Calibration



The TAT concentrations can be read directly from the reference curve via their respective absorbance values. Samples which yield absorbances above the uppermost standard must be retested in other dilutions. Sample dilutions (e.g. 1:10) must be prepared using a normal plasma with a TAT content of < 4 µg/L. The TAT content of the normal plasma must be taken into account in the calculation of the TAT concentration of the sample.

Internal Quality Control

Quality control is accomplished for each series of measurements using the CONTROL supplied in the kit. The measurement values for the samples can only be used if the value for the control is within the confidence interval.

Limitations of the Procedure

Hemolytic, lipemic and rheumatoid factors-containing plasmas do not interfere with the determination.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Note: Incorrect blood collection technique, e.g. inadequate mixing of the sample and citrate solution, can lead to falsely elevated TAT values.

Reference Intervals

In a study of ostensibly healthy individuals using a specific lot of Enzygnost® TAT micro, the following values were obtained:

	n	Median	95 % Reference Interval 2.5^{th}–97.5^{th} Percentile
Citrated plasma of healthy adults	196	< 2.0 µg/L	< 2.0–4.2 µg/L

Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

Specific Performance Characteristics

Note: The values cited for specific performance characteristics of the assay represent typical results and are not to be regarded as specifications for the Enzygnost® TAT micro test kit.

Measuring Range

The measurement range of Enzygnost® TAT micro extends from 2.0 to 60 µg/L.

Specificity

The test is specific for human thrombin/antithrombin III complex.

Precision

In the range between 2 and 60 µg/L the coefficient of variation within series (intra-assay CV) was found to fall between 4 and 6 % whereas the coefficient of variation from day to day (inter-assay CV) was found to fall between 6 and 9 %. The recovery of TAT in the plasma was 95 to 105 %.

The reproducibility was assessed by Siemens Healthineers for Enzygnost TAT micro based on variance of the sensitivity of eleven (11) production lots. The lot-to-lot variability was found to be < 16 % (samples < 6 µg/L); < 13 % (samples between 6 and 20 µg/L); < 11 % (samples > 20 µg/L).

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Current Version of Applicable Version of electronic Instructions for Use

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile	CE	CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

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