Catalog # HTPAKT-TOT

Human tPA Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Human tPA total antigen assay is intended for the quantitative determination of total tissue plasminogen activator in human plasma.

BACKGROUND

Tissue plasminogen activator is a serine protease that catalyzes the activation of plasminogen to plasmin [1]. Clinical studies have indicated that high tPA levels may increase the risk for thrombosis [2], whereas decreased levels may cause neuronal plasticity and degeneration [3].

ASSAY PRINCIPLE

tPA present in plasma reacts with the capture antibody coated on the microtiter plate. Free, latent and complexed tPA will bind to the plate. After appropriate washing steps, polyclonal anti-human tPA primary antibody is added. Excess primary antibody is washed away and bound antibody, which is proportional to the original total tPA present in the samples, is then reacted with the horseradish peroxidase secondary antibody. Following an additional washing step, TMB is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of total tPA in the sample.

REAGENTS PROVIDED

♦ **Plate:**
1-96 well immulon plate (8x12 removable wells) coated, blocked, and dried with capture antibody

♦ **10X Wash Buffer:**
1 bottle of 50ml wash; bring to 1X using DI water

♦ **Human tPA activity standard:**
1 vial lyophilized standard

♦ **Anti-human tPA primary antibody:**
1 vial lyophilized polyclonal anti-human tPA antibody

♦ **Anti-rabbit horseradish peroxidase secondary antibody:**
1 vial concentrated HRP labeled antibody

♦ **TMB substrate solution:**
1 bottle of 10ml solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer

•3% Blocking buffer
•Microtiter plate spectrophotometer operable at 450nm
•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning - The tPA standards are of human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV.
Since no tests are currently available to assure that no infectious agents are present, the standard must be treated as is recommended at the Biosafety Level 2 as potentially infectious human serum or blood specimen in the Centres for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

• **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
• **DO NOT** pipette reagents by mouth.
• Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
• Keep plate covered except when adding reagents, washing, or reading.
• **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

•TBS buffer: **0.10M TRIS, 0.15M NaCl, pH 7.4**
•**Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate [6]. This insures that the fast-acting inhibitor for single-chain tPA which is usually present in large excess, is inhibited from quenching tPA activity [5]. Immediately after collection of blood, samples must be centrifuged at 2500Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The tPA activity samples collected in the Stabilyte™ media is stable for up to 5 hours or frozen at –20°C for up to one month or up to 5 months at -70°C.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute standard as directed on vial to give a 1,000 ng/ml standard solution.
Dilution table for preparation of human tPA standard:

tPA concentration (ng/ml)	Dilutions
10	990µl (BSA) + 10µl (from vial)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0.05	500µl (BSA) + 500µl (0.1ng/ml)
0.02	600µl (BSA) + 400µl (0.05ng/ml)
0	500µl (BSA) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100µl tPA standards (enough for duplicates) and unknowns to wells. Carefully record the position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.
NOTE: If the unknown is thought to have high tPA levels, dilutions may be made in 3% BSA blocking buffer.

Primary Antibody Addition:
Reconstitute primary antibody by adding 10ml blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

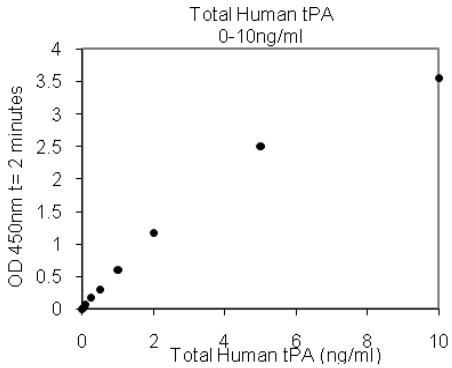
Secondary Antibody Addition:
Dilute 1µl conjugated secondary antibody in 10ml BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

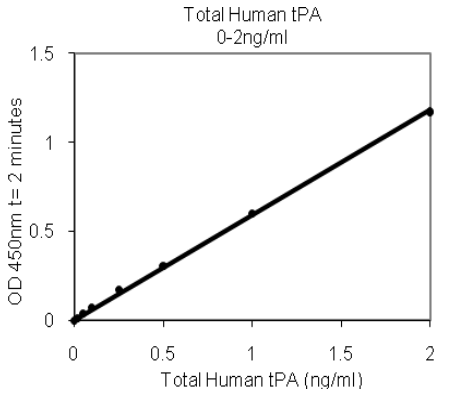
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of tPA in the standards. Fit a straight line through the points using a linear fit procedure. The tPA activity in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





EXPECTED VALUES

The basal level of tPA in healthy males and females, age 25-34 years, were found to be 5.5ng/ml and 4.0ng/ml respectively. tPA antigen increases with age; males and females age 55-64 years the median levels are 8.6ng/ml and 7.6ng/ml respectively [9,10].

Abnormalities in tPA levels have been reported in the following conditions:

♦ Neuronal plasticity and degeneration: Decreased levels of tPA have been implicated in the process of neuronal plasticity and degeneration [1,3].

♦ Arthritis: Decreased tPA levels may exacerbate arthritis [4].

♦ Deep venous thrombosis: Increased tPA levels may contribute to deep venous thrombosis [2].

♦ Coronary heart disease: Increased tPA levels may contribute to severe coronary heart disease [2].

♦ Pregnancy: Increased tPA levels are observed during pregnancy [7].

♦ Myocardial infarction: Increased tPA levels are observed in men who suffered MI compared to the matched controls[11].

♦ Stroke: Increased tPA levels have been associated with future stroke [12].

PERFORMANCE CHARACTERISTICS

The assay measures total tPA in the 0.02-10 ng/ml range. Samples giving tPA levels above 10 ng/ml should be diluted in 3% BSA blocking buffer.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCE

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Example of ELISA Kit Plate Layout

96 Well Plate

Standards: 18 wells **Samples:** 78 well

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.02ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml		
B	0	0.02ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml		
C												
D												
E												
F												
G												
H												