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Human tPA Total Antigen ELISA Kit

Catalog # HTPAKT-TOT

Strip well format. Reagents for up to 96 tests.

Rev: May 2017

INTENDED USE

This human tissue-type plasminogen activator (tPA) total antigen assay is intended for the quantitative determination of total tPA in human plasma and other biological fluids. **For research use only.**

BACKGROUND

tPA 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

Human tPA will bind to the affinity purified capture antibody coated on the microtiter plate. Free and complexed tPA will bind to the plate. After appropriate washing steps, monoclonal anti-human tPA primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with the peroxidase conjugated secondary antibody. Following an additional washing step, TMB is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human tPA. Color development is proportional to the concentration of tPA in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human tPA antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human tPA standard:** 1 vial lyophilized standard
- **Human PAI-1/tPA depleted plasma:** 3 vials lyophilized plasma
- **Anti-human tPA primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase-conjugated secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.

- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.
- Each donor unit of human depleted plasma 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 plasma must be treated as is recommended at Biosafety Level 2 as a potentially infectious human serum or blood specimen in the U.S. Department of Health and Human Services manual, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, 2009.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1,000ng/ml standard solution.

The diluent used for the standard curve is dependent on the samples to be analyzed. Use depleted plasma for plasma and serum samples or blocking buffer for cell culture and tissue lysate samples. Reconstitute each vial of depleted plasma with 1ml deionized water. Combine 50µl of 1,000ng/ml standard with 150µl of the appropriate diluent to make a 250ng/ml intermediate.

Dilution table for preparation of human tPA standard:

tPA concentration (ng/ml)	Dilutions
25	450µl (Diluent) + 50µl (250ng/ml)
10	300µl (Diluent) + 200µl (25ng/ml)
5	250µl (Diluent) + 250µl (10ng/ml)
2	300µl (Diluent) + 200µl (5ng/ml)
1	250µl (Diluent) + 250µl (2ng/ml)
0.5	250µl (Diluent) + 250µl (1ng/ml)
0.2	300µl (Diluent) + 200µl (0.5ng/ml)
0	250µl (Diluent) 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 and add 100µl tPA standards (in duplicate) and unknowns to wells. Carefully record 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: The assay measures tPA antigen in the 0.2-25 ng/ml range. If the unknown is thought to have high tPA levels, dilutions may be made in blocking buffer. Plasma samples must be applied directly to the plate without dilution for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and 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

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of 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 2-8 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

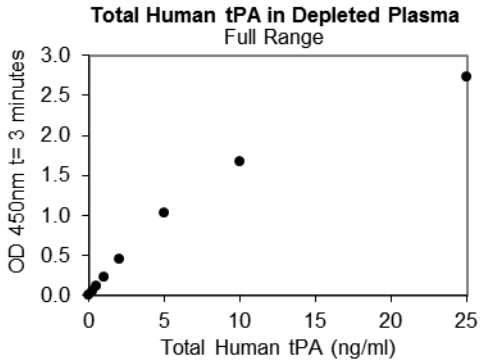
Measurement

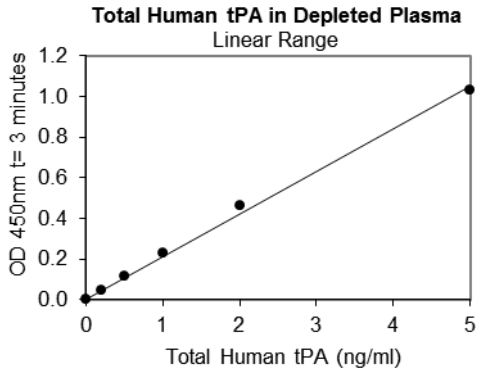
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

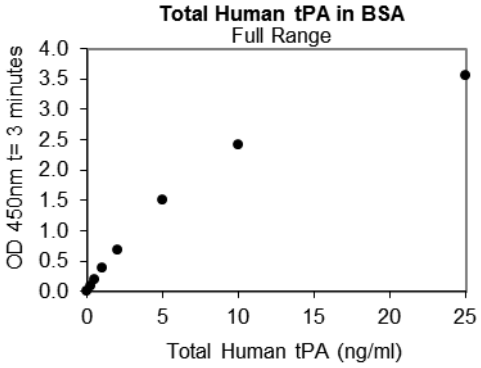
Calculation of Results

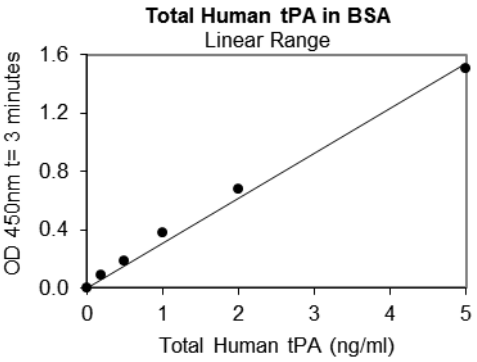
Plot A_{450} against the amount of tPA in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of tPA in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):









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

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.047-0.053 for BB, and 0.055-0.061 for tPA/PAI-1 depleted plasma) and calculating the corresponding concentration. The MDD was 0.0108ng/ml for BB and 0.0140ng/ml for tPA/PAI-1 depleted plasma.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: This assay recognizes natural and recombinant human tPA. Significant cross reaction is observed with pooled normal plasma from mouse, rat, and pig. Pooled normal plasma from rabbit, cyno monkey, rhesus monkey, guinea pig, dog and sheep were assayed for cross-reactivity. No significant cross-reactivity was observed.

REFERENCES

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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 of or contact with the above product.

Example of ELISA Plate Layout
96 Well Plate: 16 Standard wells, 80 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml				
B	0	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml				
C												
D												
E												
F												
G												
H												