Version 01-03/25

User's Manual

Human t-PA ELISA Kit

REF DEIA2167

96T

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This assay employs a quantitative enzyme immunoassay technique that measures the specified antigen in samples.

General Description

Tissue-type plasminogen activator (tPA) is a serine protease that converts the zymogen plasminogen into the active serine protease plasmin, the primary enzyme responsible for the removal of fibrin deposits. tPA is a 68 kDa glycoprotein that is synthesized by endothelial cells in normal blood vessels and displays relatively high affinity for fibrin, suggesting that it functions predominately in physiological thrombolysis in vivo.

Principles of Testing

The Human t-PA ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human t-PA in plasma, serum, urine, saliva, milk, cell culture, and tissue extract samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures t-PA in approximately 4 hours. A polyclonal antibody specific for t-PA has been precoated onto a 96-well microplate with removable strips. Human t-PA in standards and samples is sandwiched by the immobilized antibody and a biotinylated antibody specific for t-PA, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Human tPA Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against tPA. □
2. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. □
3. Human tPA Standard: Human tPA in a buffered protein base (8 ng, lyophilized). □
4. Biotinylated Human tPA Antibody (50x): A 50-fold concentrated biotinylated antibody against human tPA (120 µL)
5. MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). □
6. Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
7. Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 µL).
8. Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). □
9. Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm. □
2. Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel). □
3. Deionized or distilled reagent grade water.

Specimen Collection And Preparation

Plasma:

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Sample dilution is suggested within the range of 2x to 20x into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Sample dilution is suggested within the range of 4x to 40x into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. □

Urine:

Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. If necessary, sample dilution is suggested within the range of 2x to 10x into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. □

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. User should determine optimal dilution factor depending on application needs. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. □

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 4-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. □

Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles. □

Tissue Extracts: Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. Samples can be stored at -20°C or below for up to 3 months.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample: 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reconstitution And Storage

1. Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
2. Store SP Conjugate and Biotinylated Antibody at -20°C.
3. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
4. Unused microplate wells may be returned to the foil pouch with the desiccants and resealed. May be stored for up to 30 days in a vacuum desiccator.
5. Diluent (1x) may be stored for up to 30 days at 2-8°C.
6. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Reagent Preparation

1. Freshly dilute all reagents and bring all reagents to room temperature before use.
2. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8°C.
3. Standard Curve: Reconstitute the 8 ng of Human tPA Standard with 2 ml of MIX Diluent to generate a 4 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The standard stock solution (4 ng/ml) should be further diluted 4-fold with MIX Diluent to produce a 1 ng/ml standard working solution. Prepare duplicate or triplicate standard points by serially diluting the standard working solution (1 ng/ml) 2-fold with equal volume of MIX Diluent to produce 0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.0156 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining stock solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[tPA] (ng/ml)
P1	1 part Standard (4 ng/ml) + 3 parts MIX Diluent	1.0
P2	1 part P1 + 1 part MIX Diluent	0.5
P3	1 part P2 + 1 part MIX Diluent	0.25
P4	1 part P3 + 1 part MIX Diluent	0.125
P5	1 part P4 + 1 part MIX Diluent	0.0625
P6	1 part P5 + 1 part MIX Diluent	0.0313
P7	1 part P6 + 1 part MIX Diluent	0.0156
P8	MIX Diluent	0.0000

4. Biotinylated Human tPA Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent. The undiluted antibody should be stored at -20°C.
5. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 20-fold with reagent grade water.
6. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20°C.

Assay Procedure

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Human tPA Standard or sample per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Human tPA Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 12 minutes or till the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections.

Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Interpretation Of Results

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-a

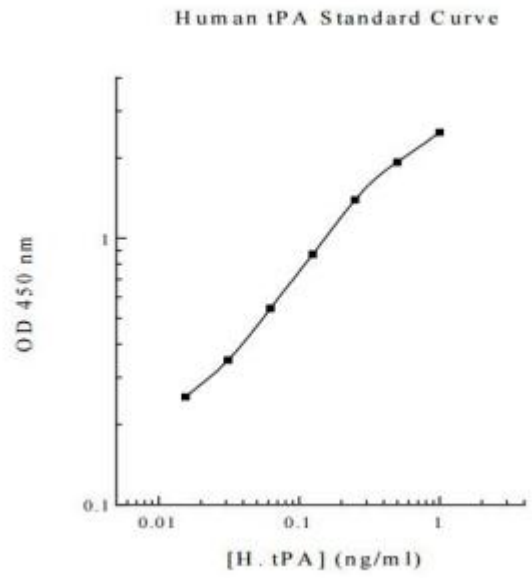
Typical Standard Curve

Typical Data

The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	1.0	2.543 2.449	2.496
P2	0.5	1.972 1.890	1.931
P3	0.25	1.400 1.386	1.393
P4	0.125	0.891 0.852	0.872
P5	0.0625	0.563 0.527	0.545
P6	0.0313	0.377 0.321	0.349
P7	0.0156	0.257 0.251	0.254
P8	0.0000	0.077 0.065	0.071
Sample: Normal, Pooled Sodium Citrate Plasma (10x)		0.794 0.791	0.793

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Values

Human plasma and serum samples from healthy adults were tested (n=20). On average, tPA level was 1.1 ng/ml.

Sample	n	Average Value (ng/ml)
Human Pooled Normal Plasma	10	0.98
Human Pooled Normal Serum	10	1.31

Performance Characteristics

1. The minimum detectable dose of tPA as calculated by 2SD from the mean of a zero standard was established to be 0.01 ng/ml. □
2. This assay recognizes single chain, two-chain, and PAI-bound human tPA. □
3. Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
4. Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.6%	4.3%	3.9%	12.1%	11.1%	8.9%
Average CV (%)	4.3%			10.7%		

Detection Range

0.031-2 ng/ml

Specificity

Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	10%
Mouse	None
Rat	None
Swine	30%
Rabbit	10%

Note: 10% FBS in culture media will not affect the assay.

Linearity

Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
2x	91%	92%
4x	99%	100%
8x	97%	101%

Recovery

Standard Added Value	0.03 – 0.5 ng/ml
Recovery %	87 – 112%
Average Recovery %	97%

Precautions

1. This product is for Research Use Only and is not intended for use in diagnostic procedures. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP Conjugate) as instructed, prior to running the assay. □
2. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. □
3. Spin down the SP Conjugate vial and the biotinylated antibody vial before opening and using contents. □
4. The Stop Solution is an acidic solution. □
5. The kit should not be used beyond the expiration date.

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