Human Total tPA ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Add 50 µl of standard/samples per well.
Incubate 2 hours.

Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.

Wash, then add 50 µl of SP per well.
Incubate 30 minutes.

Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 12 minutes.

Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Total tPA ELISA Kit

Catalog No. ET2001-1
Sample Insert/Reference Only

Introduction

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 (1). 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* (2). A high level of tPA is a good prognostic marker for breast cancer (3 - 4). tPA may minimize the formation of metastasis by preventing tumor cell adherence at sites of trauma (5). On the other hand, gastrointestinal cancer is accompanied by a decrease in tPA (6).

Principle of the Assay

The AssayMax Human Total tPA ELISA kit is designed for detection of human tPA in plasma, serum, milk, cell culture supernatants, and tissue extract. This assay employs a quantitative sandwich enzyme immunoassay technique that measures total tPA in less than 4 hours. A polyclonal antibody specific for tPA has been pre-coated onto a microplate. tPA in standards and samples is sandwiched by the immobilized antibody and a biotinylated antibody specific for tPA, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP Conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP Conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.

- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human tPA Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against tPA.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human tPA Standard:** Human tPA in a buffered protein base (10 ng, lyophilized).
- **Biotinylated Human tPA Antibody (50x):** A 50-fold concentrated biotinylated antibody against human tPA (140 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and biotinylated antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccants and resealed. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstitution.

Other Supplies Required

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

Sample Collection, Preparation and Storage

- **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. Dilute samples 1:2 into MIX Diluent and assay. 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. Dilute samples 1:2 into MIX Diluent and assay. 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 and assay. 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, measure the protein concentration, and assay. The samples can be stored at -20°C or below for up to 3 months.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 10 ng of Human tPA Standard with 1 ml of MIX Diluent to generate a stock solution of 10 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the tPA standard solution (10 ng/ml) 1:2 with equal volume of MIX Diluent to produce 5, 2.5, 1.25, 0.625, and 0.313 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[tPA] (ng/ml)
P1	1 part Standard (10 ng/ml)	10.00
P2	1 part P1 + 1 part MIX Diluent	5.000
P3	1 part P2 + 1 part MIX Diluent	2.500
P4	1 part P3 + 1 part MIX Diluent	1.250
P5	1 part P4 + 1 part MIX Diluent	0.625
P6	1 part P5 + 1 part MIX Diluent	0.313
P7	MIX Diluent	0.000

- **Biotinylated Human tPA Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- 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.
- Add 50 µl of Human tPA Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- 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.
- Add 50 µl of Biotinylated Human tPA Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.

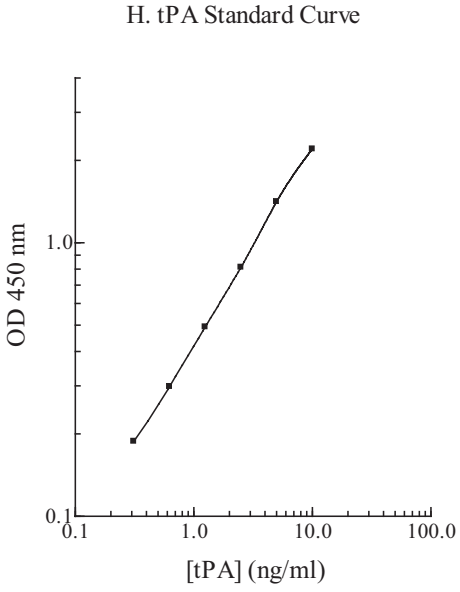
- Add 50 μl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- 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.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

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



Precision, Sensitivity and Specificity

- The minimum detectable level of tPA was typically ~ 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8% and 7.3% respectively.
- This assay recognizes single chain, two-chain, and PAI-bound human tPA.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
No Dilution	89%	92%
1:2	96%	99%
1:4	98%	101%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
No Dilution	89%
1:2	96%
1:4	102%

Recovery

Standard Added Value	0.4 – 4 ng/ml
Recovery %	87-113%
Average Recovery %	98 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	40%
Mouse	None
Rat	10%
Swine	80%
Rabbit	None

References

(1) Vassalli, J.D. *et al.* (1991) *J. Clin. Invest*. 88:1067
(2) Collen, D. and Lijnen, H.R. (1991) *Blood* 78:3114
(3) Duffy, M.J. *et al.* (1992) *Fibrinolysis* 6:55
(4) Ruppert, C. *et al.* (1997) Cancer Detect. Prev. 21:452
(5) Murthy, M.S. *et al.* (1991) *Cancer* 68: 1724
(6) Nishino, N. *et al.* (1988) *Thromb. Res.* 50:527

Version 2.0

Related products

- EP1105-1 AssayMax Human PAI-1/tPA ELISA Kit (Plasma, Serum, Milk, Cell Culture Supernatants, and Tissue samples)
- EP1100-1 AssayMax Human PAI-1 ELISA Kit (Plasma, Serum, Saliva, Cell Culture Supernatants, and Tissue samples)
- ET1001-1 AssayMax Human tPA ELISA Kit (Plasma, Serum, Urine, Saliva, Milk, and Cell Culture Supernatants)