



AssayMax Human Tissue-Type Plasminogen Activator (tPA) ELISA Kit

Catalog No. ET1001-1

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). 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).

Principal of the Assay

The AssayMax Human tPA ELISA kit is designed for detection of human tPA in plasma, urine, saliva, milk, cell culture supernatants and tissue extract. This assay employs a quantitative sandwich enzyme immunoassay technique that measures tPA in less than 4 hours. A murine 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 polyclonal 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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **tPA Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against tPA.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **tPA Standard:** Human tPA in a buffered protein base (2 ng, lyophilized).
- **Biotinylated tPA Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against human tPA (150 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 kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

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

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:20 into MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes and assay. The samples can be stored at -20⁰C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:8 into MIX Diluent. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20⁰C or below for up to 3 months. 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 min. Collect the supernatant, measure the protein concentration and assay. The samples can be stored at -20⁰C or below for up to 3 months.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:4 into MIX Diluent. The undiluted samples can be stored at -20⁰C 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):** Dilute MIX Diluent Conc. 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 2 ng of human tPA Standard with 1 ml of MIX Diluent to generate a stock solution of 2 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 (2 ng/ml) twofold with equal volume of MIX Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[tPA] (ng/ml)
P1	1 part Standard (2 ng/ml)	2.000
P2	1 part P1 + 1 part MIX Diluent	1.000
P3	1 part P2 + 1 part MIX Diluent	0.500
P4	1 part P3 + 1 part MIX Diluent	0.250
P5	1 part P4 + 1 part MIX Diluent	0.125
P6	1 part P5 + 1 part MIX Diluent	0.063
P7	1 part P6 + 1 part MIX Diluent	0.031
P8	1 part MIX Diluent	0.000

- **Biotinylated 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):** 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 desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two 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 it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated tPA Antibody to each well and incubate for one hour.
- Wash a 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 a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 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

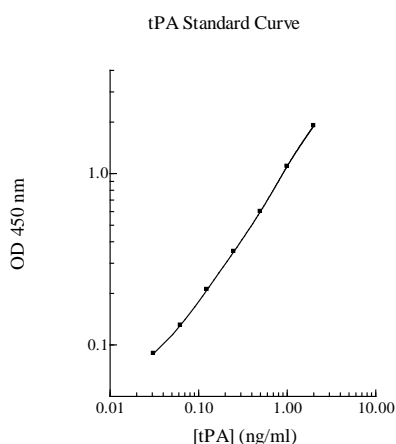
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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 less than 0.03 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.1% respectively.
- This assay recognizes single chain, two-chain, and PAI-bound human tPA.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	
1:10	101%	
1:20	103%	
1:40	107%	

	Average Percentage of Expected Value	
Sample Dilution	Saliva	Milk
No Dilution	99%	94%
1:2	101%	95%
1:4	107%	97%

	Average Percentage of Expected Value
Sample Dilution	Urine
1:2	95%
1:4	96%
1:8	101%

Recovery

Standard Added Value	0.05 – 0.5 ng/ml
Recovery %	86-114%
Average Recovery %	98 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	10%
Mouse	None
Rat	None
Swine	30%
Rabbit	10%

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

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 7.0

Related products

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