



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

Catalog Number 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, cell culture supernatants and tissue. This assay employs a quantitative sandwich enzyme immunoassay technique that measures tPA in 3.5 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 (10 ng, lyophilized)

- **Biotinylated tPA Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human tPA (80 µl)
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl)
- **MIX Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted Standard 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, 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.)
- **Urine:** Collect urine using sample pot. 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.
- **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.
- **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.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the 10 ng of human tPA Standard with 5 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 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).

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

- **MIX Diluent Concentrate (10x):** Dilute MIX Diluent Conc. 1:10 with reagent grade water.
- **Biotinylated tPA Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent.
- **Wash Buffer Concentrate (10x):** Dilute Wash Buffer Concentrate 1:10 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.

Assay Procedure

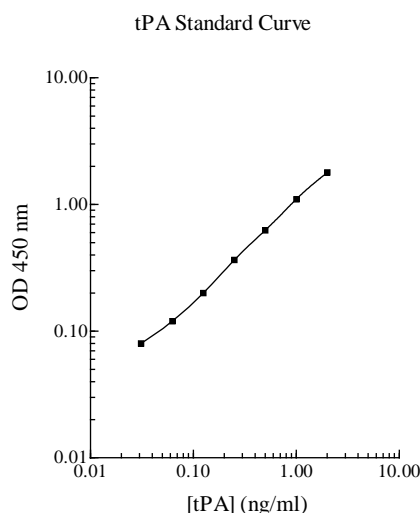
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal 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 and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated tPA Antibody to each well and incubate for 60 minutes.
- Wash five times with 200 µl of Wash Buffer.
- 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 five times with 200 µl of Wash Buffer.
- 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**. 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 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 of the linear portion of the curve using linear or log-log 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 4.6% and 6.7% respectively.
- This assay recognizes single chain, two-chain, and PAI-bound human tPA.

Cross-Reactivities

Species	% Cross Reactivity
Monkey	< 30 (suggest 1:3 for plasma sample)
Mouse	None
Rat	< 10 (suggest 1:2 for plasma sample)
Swine	< 40 (suggest 1:8 for plasma sample)
Beagle	None
Bovine	None

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

Recovery

Standard Added Value	5 – 100 ng
Recovery %	82-117 %
Average Recovery %	99.5 %

Linearity

	Average Percentage of Expected Value
Sample Dilution	Plasma
1:10	100%
1:20	105%
1:40	108%

	Average Percentage of Expected Value
Sample Dilution	Urine
No Dilution	96%
1:2	100%
1:4	108%

References

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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

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