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, 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 (5 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 (120 µl)
- **EIA 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 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted Standard at -20^{0}C 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 2,000x g for 10 minutes and assay. Dilute samples 1:20 into EIA Diluent. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x g for 10 minutes and assay. Store samples at -20^{0}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 14,000 x g for 20 min. Collect the supernatant, measure the protein concentration and assay. Freeze the remaining extract at -20^{0}C or below.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the 5 ng of human tPA Standard with 2.5 ml of EIA 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 EIA Diluent to produce 1, 0.5, 0.25, 0.125, 0.0625 and 0.0313 ng/ml. EIA 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 EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.031
P8	1 part EIA Diluent	0.000

- **EIA Diluent Concentrate (10x):** Dilute EIA 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 EIA 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 EIA 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^{0}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 complete remove liquid at each step.
- Add 50 µl of Biotinylated tPA Antibody to each well and incubate for 30 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 5 to10 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.

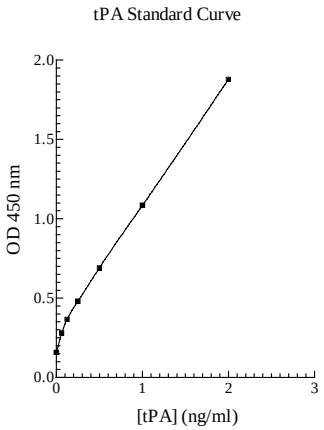
Data Analysis

- 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 of 20 for plasma analysis.

Standard Curve

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



Performance Characteristics

- The minimum detectable level of tPA was typically less than 0.015 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.9% and 7.0% respectively.
- This assay recognizes single chain, two-chain, and PAI-bound human tPA. No significant cross-reactivity or interference was observed.

References

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