Datasheet for ABIN612782

PLAT ELISA Kit

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Overview

Quantity:	96 tests
Target:	PLAT
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	0.03 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human tPA ELISA kit is designed for detection of human tPA in plasma, urine, saliva, milk, cell culture supernatants and tissue extract
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes single chain, two-chain, and PAI-bound human tPA. 10% FBS in culture media will not affect the assay.
Components:	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 (150µl). MIx Diluent Concentrate (10x): A

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	10-fold concentrated buffered protein base (30 ml). 1 Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80µl). 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).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	PLAT
Alternative Name:	Tissue-Type Plasminogen Activator (tPA) (PLAT Products)
Background:	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 (2). High level of tPA is a good prognostic marker for breast cancer. tPA may minimize the formation of metastasis by preventing tumor cell adherence at sites of trauma. On the other hand, gastrointestinal cancer is accompanied by a decrease in tPA.
Pathways:	Autophagy, Smooth Muscle Cell Migration, Platelet-derived growth Factor Receptor Signaling, SARS-CoV-2 Protein Interactome

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	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.
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 2-8°C. 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 2 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.
Sample Collection:	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 -20°C 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 -20°C 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 -20°C 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 -20°C 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 -20°C 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 -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room

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	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 3 unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	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 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.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.5% and 7.1% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened MIx Diluent may be

Handling

stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°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.

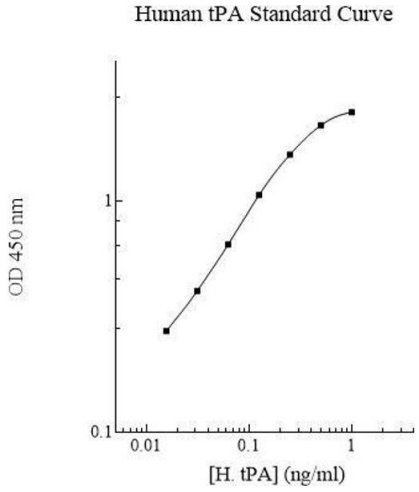
Publications

Product cited in:

Lee, Siddique, Kim, Green, Van Horn, Allison, Wassertheil-Smoller, Greenland: "Hemostatic Markers and Long-Term Risk of Intracerebral Hemorrhage in Postmenopausal Women." in: **Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association**, (2016) (PubMed).

Zhou, Lin, Wu, Xiong, Lv, Zheng, Huang, Chen: "The high-level expression of human tissue plasminogen activator in the milk of transgenic mice with hybrid gene locus strategy." in: **Molecular biotechnology**, Vol. 50, Issue 2, pp. 137-44, (2012) (PubMed).

Images



ELISA

Image 1.