Datasheet for ABIN612792

PLAU ELISA Kit

3 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	PLAU
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	20 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Urokinase (uPA) ELISA kit is designed for detection of human uPA in plasma, serum, tissue, saliva, milk, and cell culture supernatants
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes single chain, two-chain, and both receptor and PAI-bound human uPA.
Components:	uPA Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against uPA. 1 Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. uPA Standard: Human uPA in a buffered protein base (16 ng, lyophilized). Biotinylated uPA Antibody (100x): A 100-fold biotinylated polyclonal antibody against human uPA (80µl). MIx Diluent Concentrate (10x): A 10-fold buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated

Product Details

	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:	PLAU
Alternative Name:	Urokinase (uPA) (PLAU Products)
Background:	Urokinase-type plasminogen activator (uPA) is a highly restricted serine protease that converts the zymogen plasminogen to active plasmin, a broad-spectrum serine proteinase capable of degrading most of the major protein components of the extracellular matrix. Binding of uPA to its receptor and subsequent uPA mediated pericellular proteolysis are involved in many process including cell migration and tissue remodeling in angiogeenesis, atherogenesis, tumor cell metastasis, and ovulation. High level of uPA is a poor prognostic marker for aggressive breast cancer, aggressive prostate cancer, bladder cancer and gastric cancer.
Pathways:	Cellular Response to Molecule of Bacterial Origin, Carbohydrate Homeostasis, Autophagy, Smooth Muscle Cell Migration

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 urokinase in less than 4 hours. A polyclonal antibody specific for uPA has been pre-coated onto a microplate. Urokinase in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for uPA, 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. If crystals

	have formed in the concentrate, mix gently until the crystals have completely dissolved. MIx Diluent Concentrate (10x): Dilute the MIx Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 16 ng of human uPA Standard with 2 ml of MIx Diluent to generate an 8 ng/ml of stock solution. 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 uPA standard solution (8 ng/ml) twofold with equal volume of MIx Diluent to produce 4, 2, 1, 0.5 and 0.25 ng/ml solutions. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [uPA] (ng/ml) P1 1 part Standard (8 ng/ml) 8.000 P2 1 part P1 + 1 part MIx Diluent 4.000 P3 1 part P2 + 1 part MIx Diluent 2.000 P4 1 part P3 + 1 part MIx Diluent 1.000 P5 1 part P4 + 1 part MIx Diluent 0.500 P6 1 part P5 + 1 part MIx Diluent 0.250 P7 MIx Diluent 0.000 Biotinylated uPA Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute Wash Buffer Conc. 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 use supernatants. Dilute samples 1:2 with 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 anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:2 into MIx Diluent. Store serum at -20°C or below. Avoid repeated freeze-thaw cycles Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants 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 800 x g for 10 minutes. Dilute samples 1:20 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. Tissue: Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton x-100 and centrifuge at 14000 x g for 30 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 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. 2 Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10

	minutes and assay. 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 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 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. 3 Add 50 µL of Biotinylated uPA 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.
Calculation of Results:	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.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 5.2 % and 7.1% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	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. 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 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:	Cekmez, Cekmez, Ozkaya, Pirgon, Yılmaz, Yılmaz, Kaya, Süer, Küçüközkan: "uPAR, IL-33, and ST2 values as a predictor of subclinical chorioamnionitis in preterm premature rupture of membranes." in: **Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research**, Vol. 33, Issue 12, pp. 778-82, (2013) (PubMed). Wyrębska, Gach, Szemraj, Szewczyk, Hrabec, Koszuk, Janecki, Janecka: "Comparison of anti-invasive activity of parthenolide and 3-isopropyl-2-methyl-4-methyleneisoxazolidin-5-one (MZ-6)--a new compound with α-methylene-γ-lactone motif--on two breast cancer cell lines." in: **Chemical biology & drug design**, Vol. 79, Issue 1, pp. 112-20, (2012) (PubMed). Lugano, Peña, Badimon, Padró: "Aggregated low density lipoprotein induce impairment of the cytoskeleton dynamics through UPA/UPAR in human VSMC." in: **Journal of thrombosis and haemostasis : JTH**, (2012) (PubMed).