

Datasheet for ABIN612673

PLG ELISA Kit

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Overview

Quantity: 96 tests

Target: PLG

Reactivity: Cow

Method Type: Sandwich ELISA

Minimum Detection Limit: 3 ng/mL

Application: ELISA

Product Details

Purpose: The AssayMax Bovine Plasminogen ELISA kit is designed for detection of bovine plasminogen in urine and cell culture supernatants

Brand: AssayMax

Sample Type: Cell Culture Supernatant

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: This assay recognizes both natural and recombinant bovine plasminogen.

Components: Plasminogen Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against bovine plasminogen. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. 1 Plasminogen Standard: Bovine plasminogen in a buffered protein base (200 ng, lyophilized). Biotinylated Plasminogen Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against bovine plasminogen (80µl). EIA Diluent Concentrate (10x): A 10-fold

Product Details

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 (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, 200-1000µL and multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	PLG
Alternative Name:	Plasminogen (PLG Products)
Background:	Plasminogen is a single chain glycoprotein zymogen that is synthesized in the liver and circulated in plasma with a molecular weight of 90 kDa. The N-terminal portion of the molecule is made up of five kringle domains that bind to fibrin. The native molecule has an amino-terminal glutamic acid, known as glu-plasminogen, but this can undergo proteolytic cleavage by plasmin to lys-plasminogen. The inactive proenzyme plasminogen is converted to the active enzyme plasmin that ultimately digests fibrin. Tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) catalyzes the activation of plasminogen, while plasminogen activator inhibitors (PAIs) inhibits the activation. The plasminogen system plays a role in macrophage recruitment, arterial stenosis, atherosclerosis, aneurysm formation, skin and corneal wound healing, glomerulonephritis, and neovascularization.
Pathways:	Complement System , Lipid Metabolism

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 plasminogen in less than 4 hours. A polyclonal antibody specific for plasminogen has been pre-coated onto a 96-well microplate with removable strips. Plasminogen in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for plasminogen, 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. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 200 ng of bovine Plasminogen Standard with 1 ml of EIA Diluent to produce 200 ng/ml of 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 standard solution (200 ng/ml) 1:2 with equal volume of EIA Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.125 ng/ml of solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Plasminogen] (ng/ml) P1 Standard (200 ng/ml) 200.000 P2 1 part P1 + 1 part EIA Diluent 100.000 P3 1 part P2 + 1 part EIA Diluent 50.000 P4 1 part P3 + 1 part EIA Diluent 25.000 P5 1 part P4 + 1 part EIA Diluent 12.500 P6 1 part P5 + 1 part EIA Diluent 6.250 P7 3.125 1 part P6 + 1 part EIA Diluent P8 EIA Diluent 0.000 Biotinylated Plasminogen Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the 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 EIA Diluent. Any remaining solution should be frozen at -20°C.

Sample Collection: Cell Culture Supernatants: Collect cell culture media and centrifuge at 2000 x g for 10 minutes to remove debris. Dilute cell culture media into EIA Diluent. The user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples 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 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 Plasminogen 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 12 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 4.9 % and 7.2 % respectively.
Restrictions:	For Research Use only

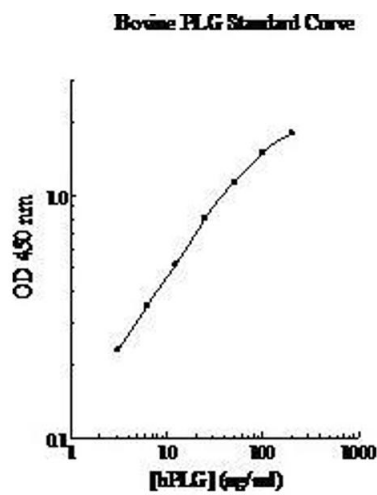
Handling

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 components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for

Handling

up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C.
Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Images



ELISA

Image 1.