Datasheet for ABIN612728

LYZ ELISA Kit

1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	LYZ
Reactivity:	Human
Method Type:	Competition ELISA
Minimum Detection Limit:	50 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human Lysozyme ELISA kit is designed for detection of Lysozyme in detection of human plasma, serum, urine, salvia, milk, other body fluids and cell culture supernatant
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant, Milk
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes both natural and recombinant human Lysozyme.
Components:	µlysozyme Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Lysozyme. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay. 1 µlysozyme Standard: Human Lysozyme in a buffered protein base (50 ng, lyophilized). Biotinylated Lysozyme: 1 vial, lyophilized. EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered

Product Details

	surfactant (30 ml). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (90µl). 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:	LYZ
Alternative Name:	Lysozyme (LYZ Products)
Background:	Lysozyme is one of the anti-microbial agents found in human milk, and is also present in spleen, lung, kidney, white blood cells, plasma, saliva, and tears. Lysozyme has 130 amino acids and its natural substrate is the bacterial cell wall peptidoglycan. Since synthesized by granulocytes and macrophages, lysozyme can act as a useful marker for myelomonocytic cells. Increased levels of lysozyme in urine and serum are diagnostic indicators for acute monocytic leukemia and acute myelomonycytic leukemia. Elevated lysozyme levels were found in synovial fluids of the inflammatory arthritides and osteoarthritis. Human lysozyme gene mutations cause hereditary systemic amyloidosis. The extracellular clusterin potently inhibits human lysozyme amyloid formation by interacting with prefibrillar species. Salivary lysozyme, a marker for oral infection and hyperglycemia, might display a significant relationship with hypertension, an early stage of cardiovascular disease.

Application Details

Sample Volume:	25 µL
Assay Time:	< 3 h
Plate:	Pre-coated
Protocol:	This assay employs a quantitative sandwich enzyme immunoassay technique that measures Lysozyme in less than 3 hours. A polyclonal antibody specific for Lysozyme has been pre-coated onto a microplate. Lysozyme in standards and samples is competed by a biotinylated Lysozyme sandwiched by the immobilized antibody and 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.

Application Details

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 MIx Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Lysozyme Standard: Reconstitute the 6 g of human Lysozyme Standard with 1 ml of EIA Diluent to generate a standard solution of 6 g/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (6 g/ml) twofold with equal volume of EIA Diluent to produce 3, 1.5, 0.75, 0.375, 0.187 and 0.093 g/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [Lysozyme] (g/ml) P1 1 part Standard (6 g/ml) 6.000 P2 1 part P1 + 1 part MIx Diluent 3.000 P3 1 part P2 + 1 part MIx Diluent 1.500 P4 1 part P3 + 1 part MIx Diluent 0.750 P5 1 part P4 + 1 part MIx Diluent 0.375 P6 1 part P5 + 1 part MIx Diluent 0.190 P7 1 part P5 + 1 part MIx Diluent 0.090 P8 MIx Diluent 0.000 Biotinylated Lysozyme (4x): Dilute Biotinylated Lysozyme with 4 ml EIA Diluent to produce a 4-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:4 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 MIx Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Milk: Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:1000 into EIA Diluent. Store samples at -20°C or below for up to one month. 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 25 µL of standard and/or sample per well, and immediately add 25 µL of Biotinylated Lysozyme to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for two hours at room temperature. 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 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 plate to ensure thorough mixing and

Application Details

	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.
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 4.8 % and 7.4% 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 EIA 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:	Wu, Lin, Xi, Shao, Zhou, Liu, Chen: "The development of transgenic mice for the expression of large amounts of human lysozyme in milk." in: **Biotechnology letters**, Vol. 36, Issue 6, pp. 1197-202, (2014) (PubMed).