



AssayMax Human Lysozyme ELISA Kit

Catalog # EL3010-1

Introduction

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 (1, 2). Increased levels of lysozyme in urine and serum are diagnostic indicators for acute monocytic leukemia and acute myelomonocytic leukemia (3). Elevated lysozyme levels were found in synovial fluids of the inflammatory arthritides and osteoarthritis (4). Human lysozyme gene mutations cause hereditary systemic amyloidosis (5, 6). The extracellular clusterin potentially inhibits human lysozyme amyloid formation by interacting with prefibrillar species (7). Salivary lysozyme, a marker for oral infection and hyperglycemia, might display a significant relationship with hypertension, an early stage of cardiovascular disease (8).

Principal of the Assay

The AssayMax Human Lysozyme ELISA kit is designed for detection of Lysozyme in detection of human plasma, serum, urine, saliva, milk, other body fluids and cell culture supernatant. This assay employs a quantitative sandwich enzyme immunoassay technique that measures Lysozyme in 4 hours. A polyclonal antibody specific for Lysozyme has been pre-coated onto a microplate. Lysozyme in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Lysozyme, 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

- **Lysozyme 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.
- **Lysozyme Standard:** Recombinant human Lysozyme in a buffered protein base (50 ng, lyophilized).

- **Biotinylated Lysozyme Antibody (100x):** A 100-fold biotinylated polyclonal antibody against Lysozyme (80 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **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).

Storage Condition

- Store unopened kit at 2 - 8⁰C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C 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, 200-1000µl 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 2000 x g for 10 minutes and assay. Dilute samples 1:1000 into MIX Diluent. The undiluted samples can be stored at -20⁰C 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 1:1000 into MIX Diluent. Store serum at -20⁰C 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. Store the remaining samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.
- **Saliva:** Rinse your mouth at least twice with cool water. Then chew sugarless gum for 1-2 minutes, swallowing saliva as usual. Dilute samples 1:8000 into MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to one month. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:10000 into MIX Diluent. Store samples at -20⁰C or below for up to one month. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:10 into MIX Diluent. Store samples at -20⁰C or below for up to one month. Avoid repeated freeze-thaw cycles.

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 Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8°C.
- **Lysozyme Standard:** Reconstitute the 50 ng of human Lysozyme Standard with 5 ml of MIX Diluent to generate a standard solution of 10 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 Standard solution (10 ng/ml) twofold with equal volume of MIX Diluent to produce 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Lysozyme] (ng/ml)
P1	1 part Standard (10 ng/ml)	10.000
P2	1 part P1 + 1 part MIX Diluent	5.000
P3	1 part P2 + 1 part MIX Diluent	2.500
P4	1 part P3 + 1 part MIX Diluent	1.250
P5	1 part P4 + 1 part MIX Diluent	0.625
P6	1 part P5 + 1 part MIX Diluent	0.313
P7	1 part P6 + 1 part MIX Diluent	0.156
P8	MIX Diluent	0.000

- **Biotinylated Lysozyme Antibody (100x):** Spin down the biotinylated 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 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.

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. 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 Biotinylated Lysozyme Antibody to each well and incubate for one hour.
- 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.

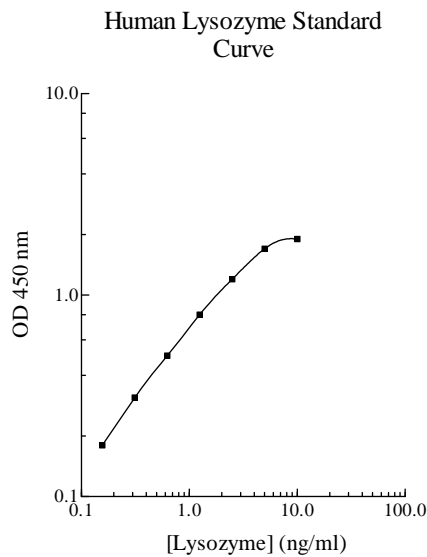
- Add 50 μ l of Chromogen Substrate per well and incubate for about 8 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to 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 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.



Performance Characteristics

- The minimum detectable dose of Lysozyme is typically 0.1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1 % and 7.2% respectively.
- This assay recognizes both natural and recombinant human Lysozyme.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:500	98%	101%
1:1000	102%	98%
1:2000	100%	102%

Sample Dilution	Average Percentage of Expected Value
	Saliva
1:4000	98%
1:8000	101%
1:16000	102%

Sample Dilution	Average Percentage of Expected Value
	Urine
1:10	106%
1:20	98%
1:40	98%

Recovery

Standard Added Value	0.5 – 5 ng/ml
Recovery %	91-113 %
Average Recovery %	102 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	<1
Mouse	None
Rat	None
Swine	<10 (suggest 1:10 dilution for plasma/serum)

References

- (1) Chung LP et al. (1988) Proc. Natl. Acad. Sci. USA 85:6227-6231
- (2) Lollike K et al. (1995) Leukemia 9:159-164
- (3) Osserman EF and Lawlor DF (1966) J.Exp. Med. 124:921-952
- (4) Bennett RM and Skosey JL (1977) Arthritis Rheum. 20:84-90
- (5) Pepys MB et al. (1993) Nature 362: 553-557
- (6) Moraitakis G and Goodfellow JM (2003) Biophy. J. 84:2149-2158
- (7) Kumita JR et al. (2007) J. Mol. Biol. 369:157-167
- (8) Ovarnstrom M et al. (2008) J. Dent. Res. 87:480-484

Version 1.3