



# **AssayMax Human Lysozyme ELISA Kit (Milk)**

Catalog No. EL3020-1

## **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 potently 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 milk. This assay employs a quantitative, competitive 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 with 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

## **Caution and Warning**

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, 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 before opening and using contents.**
- 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:** Human Lysozyme in a buffered protein base (6 µg, 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 surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 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 components of the kit at 2-8<sup>0</sup>C or -20<sup>0</sup>C upon arrival up to the expiration date.
- Store SP Conjugate at -20<sup>0</sup>C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8<sup>0</sup>C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8<sup>0</sup>C.
- Store Standard and Biotinylated Protein at 2-8<sup>0</sup>C before reconstituting with Diluent and at -20<sup>0</sup>C after reconstituting with Diluent.

## 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

- **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<sup>0</sup>C 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.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8<sup>0</sup>C.
- **Lysozyme Standard:** Reconstitute the 6 µg of human Lysozyme Standard with 1 ml of EIA Diluent to generate a standard solution of 6 µg/ml. 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 (6 µg/ml) twofold with equal volume of EIA

Diluent to produce 3, 1.5, 0.75, 0.375, 0.187 and 0.093 µg/ml. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Lysozyme] (µg/ml)
P1	1 part Standard (6 µg/ml)	6.000
P2	1 part P1 + 1 part EIA Diluent	3.000
P3	1 part P2 + 1 part EIA Diluent	1.500
P4	1 part P3 + 1 part EIA Diluent	0.750
P5	1 part P4 + 1 part EIA Diluent	0.375
P6	1 part P5 + 1 part EIA Diluent	0.187
P7	1 part P6 + 1 part EIA Diluent	0.093
P8	EIA 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):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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.

## Assay Procedure

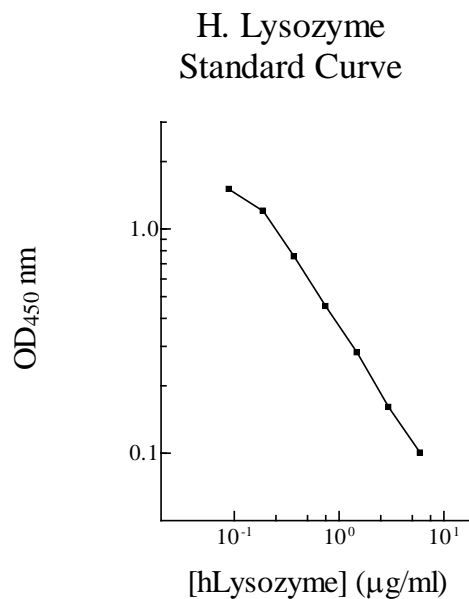
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal 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 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 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 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. 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 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.



## Performance Characteristics

- The minimum detectable dose of Lysozyme is typically ~ 0.09 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.4% respectively.
- This assay recognizes both natural and recombinant human Lysozyme.

## Linearity

	Average Percentage of Expected Value
<b>Sample Dilution</b>	<b>Milk</b>
<b>1:500</b>	99%
<b>1:1000</b>	102%
<b>1:2000</b>	101%

## Recovery

<b>Standard Added Value</b>	0.3 – 3 µg/ml
<b>Recovery %</b>	91-113 %
<b>Average Recovery %</b>	102 %

## Cross-Reactivity

<b>Species</b>	<b>% Cross Reactivity</b>
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 2.1R

## Related Products

- EL3010-1 AssayMax Human Lysozyme ELISA Kit (Plasma, Serum, Saliva, Cell Culture Supernatants and Urine samples)