Human Lysozyme ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

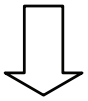
The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

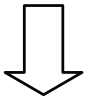
Thank you for choosing Assaypro.

Assay Summary

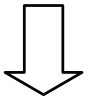
Add 50 µl of standard/sample per well.
Incubate 2 hours.



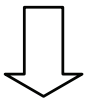
Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 1 hour.



Wash, then add 50 µl of SP per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 15 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
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AssayMax Human Lysozyme ELISA Kit

Catalog No. EL3010-1
Sample Insert/Reference Only

Introduction

Lysozyme is one of the anti-microbial agents found in human milk and is also present in the spleen, lungs, kidneys, white blood cells, plasma, saliva, and tears. Lysozyme has 130 amino acids and its natural substrate is the bacterial cell wall peptidoglycan. Since it is 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).

Principle of the Assay

The AssayMax Human Lysozyme ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of lysozyme in human plasma, serum, urine, saliva, and cell culture supernatant. This assay employs a quantitative sandwich enzyme immunoassay technique that measures lysozyme in less than 4 hours. A polyclonal antibody specific for lysozyme has been pre-coated onto a 96-well microplate with removable strips. 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

- **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.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human 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 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human Lysozyme Standard:** Human lysozyme in a buffered protein base (100 ng, lyophilized).
- **Biotinylated Human Lysozyme Antibody (50x):** A 50-fold biotinylated polyclonal antibody against lysozyme (140 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, 2 bottles).
- **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°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.
- Unused microplate wells may be returned to the foil pouch with the desiccants and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl 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 3000 x *g* for 10 minutes. Dilute samples 1:1000 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 (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:1000 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.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect saliva using samples tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:8000 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.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:10 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.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x)**: If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Human Lysozyme Standard:** Reconstitute the 100 ng of Human Lysozyme Standard with 5 ml of MIX Diluent to generate a standard solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Dilute standard (20 ng/ml) 1:4 with MIX Diluent to generate a standard solution of 5 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (5

ng/ml) 1:2 with MIX Diluent to produce 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Lysozyme] (ng/ml)
P1	1 part Standard (20 ng/ml) + 3 part MIX Diluent	5.000
P2	1 part P1 + 1 part MIX Diluent	2.500
P3	1 part P2 + 1 part MIX Diluent	1.250
P4	1 part P3 + 1 part MIX Diluent	0.625
P5	1 part P4 + 1 part MIX Diluent	0.313
P6	1 part P5 + 1 part MIX Diluent	0.156
P7	MIX Diluent	0.000

- **Biotinylated Human Lysozyme Antibody (50x):** Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 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 (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Lysozyme Standard or sample per well. Cover wells with a sealing tape and incubate for 2 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 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Lysozyme Antibody to each well and incubate for 1 hour.

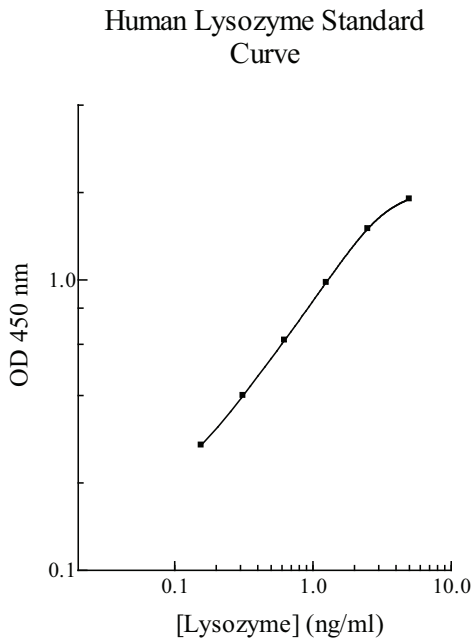
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for about 15 minutes or until 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. 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.

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 ~ 0.15 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.1% respectively.
- This assay recognizes both natural and recombinant human lysozyme.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:500	92%	91%
1:1000	99%	98%
1:2000	104%	105%

	Average Percentage of Expected Value
Sample Dilution	**Saliva**
1:4000	84%
1:8000	97%
1:16000	104%

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:5	82%
1:10	98%
1:20	101%

Recovery

Standard Added Value	0.25 – 2.5 ng/ml
Recovery %	86-112%
Average Recovery %	97%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	<5%
Monkey	<5%
Mouse	None
Rat	None
Swine	<5%
Rabbit	None
Human	100%

Reference Value

- Normal human lysozyme plasma levels range from 1 to 11 μg/ml.

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 2.2

Related Products

- EL3020-1 AssayMax Human Lysozyme ELISA Kit (Milk and Cell Culture Samples)