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Lysozyme ELISA Kit

rev. 10/16

(Catalog # K4243-100, 100 assays, Store at 4°C)

I. Introduction:

Lysozymes, also known as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk, and mucus. It is also present in cytoplasmic granules of the macrophages and the polymorphonuclear neutrophils (PMNs). BioVision's Lysozyme ELISA kit is a sandwich ELISA assay for the quantitative measurement of human Lysozyme in serum, plasma and biological fluid. The density of color is proportional to the amount of human Lysozyme captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Lysozyme.

III. Specificity:

Human

IV. Sample Type:

Serum and Biological fluid

V. Kit Contents:

Components	K4243-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4243-100-1
Lysozyme Standards (S1 – S7)	250 µl X 7	K4243-100-2
Lysozyme Controls (C1, C2)	250 µl X 2	K4243-100-3
Anti-Lysozyme Enzyme Conjugate	12 ml	K4243-100-4
Sample Diluent	20 ml X 2	K4243-100-5
TMB Substrate	12 ml	K4243-100-6
Wash Concentrate (20X)	25 ml	K4243-100-7
Stop Solution	12 ml	K4243-100-8

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C. Keep Standard <-20°C, -80°C for long term storage.

VIII. Reagents and Samples Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **20X Wash Buffer Concentrate**: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).
2. **Lysozyme Standards:** All standards are ready to use. Check standard value on each vial. This value might vary from lot to lot.
3. **Lysozyme Controls:** All controls are ready to use. Check control value on each vial.
4. **Sample Preparation:** Collect blood specimens and separate the serum immediately. Typically, specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Dilute serum samples 1:250 in sample diluent. Dilute stool samples 1:100 in sample diluent.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 25µl of the **standards**, **controls** and **diluted samples** into the assigned well.
3. Add 100µl of **anti-lysozyme enzyme conjugate** solution into all wells.
4. Incubate the plate for 60 minutes at room temperature, with shaking.

FOR RESEARCH USE ONLY! Not to be used on humans.

5. Remove liquid from all wells. Wash wells three times with 300 of 1X **wash buffer**. Blot on absorbent paper towels.
6. Add 100µl of **TMB substrate** solution to all wells
7. Incubate the plate for 15 minutes at room temperature.
8. Add 50µl of **Stop solution** to each well and gently mix for 15-20 seconds.
9. Read the absorbance on ELISA Reader of each well at 450nm within 15 minutes after adding the stop solution.

X. CALCULATION:

Check Lysozyme standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. To construct the standard curve, plot the absorbance for Lysozyme standards (vertical axis) versus Lysozyme standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

	OD 450 nm	Conc. ng/mL
Std 1	0.078	0
Std 2	0.18	1.25
Std 3	0.306	2.5
Std 4	0.600	5
Std 5	1.066	10
Std 6	1.710	20
Std 7	2.532	40

XI. RELATED PRODUCTS:

- Lysozyme Activity Assay Kit (Fluorometric) (Cat. No. K236-100)
- Lysozyme Antibody (Cat. No. A1110-100)
- Lysozyme Inhibitor Screening Kit (Cat. No. K237-100)
- EZLys™ Lysozyme, Human (Cat. No. 8009-1G, 5G)