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Product Information

Lysozyme ELISA kit

Catalog Number: EA100880
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Lysozyme ELISA Kit is intended for the quantitative measurement of lysozyme in human serum or stool.

Background

Lysozyme (muramidase) is an enzyme present in serum, plasma, amniotic fluid, stool, saliva, tears, urine and other biological fluids. Elevated lysozyme levels in urine and serum have been reported in many human disease states, including Crohn's disease, leukemias (FAB-M4, CMML, CML), tuberculosis, megaloblastic anemias, acute bacterial infections, ulcerative colitis, severe renal insufficiency, pyelonephritis, nephrosis and renal transplant rejection.

Principle of the Test

The Lysozyme kit is a solid phase direct ELISA sandwich method. The samples and the working anti-lysozyme enzyme conjugate are added to the wells coated with anti-lysozyme monoclonal antibody. Lysozyme in the patient's sample is bound to the monoclonal capture antibody and detected with a polyclonal detection antibody. Unbound lysozyme and anti-lysozyme enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of Lysozyme in the samples. A standard curve is generated relating color intensity to the concentration of Lysozyme.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell plate coated with anti-Lysozyme Monoclonal Ab	12x8x1
2. Lysozyme Standard: 7 vials (ready to use)	0.25 ml
3. Lysozyme Controls: 2 vials (ready to use)	0.25 ml
4. Anti-Lysozyme Enzyme Conjugate: 1 vial (Ready to use)	12 ml
5. Sample Diluent (ready to use)	40 ml
6. TMB Substrate: 1 bottle (ready to use)	12 ml
7. Stop Solution: 1 bottle (ready to use)	12 ml
8. 20X Wash concentrate: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water

2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. 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.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

1. **Samples:** Dilute serum samples 1:250 in sample diluent. Dilute stool samples 1:100 in sample diluent.
2. **Wash 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 (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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 25 µl of the standards, controls and diluted samples into the assigned well.
3. Add 100 µl of anti-lysozyme enzyme conjugate solution into all wells.
4. Incubate the plate for 60 minutes at room temperature, with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate solution to all wells
7. Incubate the plate for 15 minutes at room temperature.
8. Add 50 µl 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.

Calculation of Results

The standard curve is constructed as follows:

1. 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. See example of the standard attached.

2. 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.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	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

Sensitivity

The sensitivity is 0.021 ng/ml.

References

1. Agharanya JC. Clinical usefulness of ELISA technique in the assessment of thyroid function. West Afr J Med 1990;9(4):258-63.
2. Hankiewiz, J. and Swierczuk, E. 1974. Lysozymes in Human Body Fluids. Clinica Chemica Acta, 57: 205-209.
3. Meyor, K., Gelhorn, A., Prudden, J.F., et al. 1948. Lysozyme Activity in Ulcerative Alimentary Diseases. American Journal of Medicine, 5: 496-502.
4. Prockup, D.J. and Davidson, W.D., 1964. A Study of Urinary and Serum Lysozyme in Patients with Renal Disease, New England Journal of Medicine, 270: 269.

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