BOSTER

antibody and ELISA experts

Human Lysozyme OneStep ELISA Kit

Catalog number: EK7068

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Human Lysozyme OneStep ELISA Kit

Catalog Number: EK7068

Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 1 ng/ml

Assay Range: 1-40 ng/ml

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

The Bosterbio OneStep ELISA kit is a solid phase sandwich ELISA kit. Instead of incubating with standards, samples and controls and with enzyme conjugated detection antibody separately, the OneStep ELISA kit allows the user to add standards, samples and controls to wells without incubation, and an enzyme conjugate reagent is added into each well immediately. Then incubate standards, samples, controls and enzyme conjugate reagent together. After the excess enzyme conjugate is washed out, the substrate is added into each well. The enzyme catalyzes the substrate yielding a blue color (Amax = 370nm and 652nm) that changes to yellow (Amax = 450nm) upon addition of a sulfuric or phosphoric acid stop solution. The intensity of color developed is directly proportional to the concentration of target protein in the samples. A standard curve is generated relating color intensity to the concentration of target protein.

Kit Components

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

Standard Concentrations and example data

	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	0.6	5

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Std 5	1.066	10
Std 6	1.71	20
Std 7	2.532	40

Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum immediately.
2. 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.
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 (20-25°C).

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ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).
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 25ul of the standards, controls and diluted samples into the assigned well.
3. Add 100ul 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 100ul of TMB substrate solution to all wells
7. Incubate the plate for 15 minutes at room temperature.
8. Add 50ul 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

1. Reliable And Reproducible Results Will Be Obtained When The Assay Procedure Is Carried Out With A Complete
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 the FSH 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.

LIMITATION OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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