Human Myoglobin OneStep ELISA Kit

Catalog number: EK7069

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 Myoglobin OneStep ELISA Kit

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Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 25 ng/ml

Assay Range: 25-1000 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 coated with murine monoclonal anti-myoglobin.	12x8x1
2. Reference Standard Set	0.5 ml
3. Sample Diluent	25 ml
4. Enzyme Conjugate Reagent	22 ml
5. TMB Reagent	11 ml
6. Stop Solution	11 ml
7. Wash Concentrate 20x: 1 Bottle	25 ml

Standard Concentrations and example data

	OD 450 nm	Conc. ng/mL
Std 1	0.045	0
Std 2	0.191	25
Std 3	0.628	100
Std 4	1.445	250
Std 5	2.178	500

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Std 6	2.896	1000

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. 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, as 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
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
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. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

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

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).

REAGENT PREPARATION

1. All reagents should be brought to room temperature (20-25°C) before use.
2. Patient serum and control serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and

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mix 20ul serum with 180ul (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS--THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD

3. Samples with expected myoglobin concentrations over 1000 ng/ml may be quantitated by further dilution 10-fold with sample diluent.

4. 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).

ASSAY PROCEDURE

1. Patient serum and control serum should be diluted 10 fold before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20ul serum or plasma with 180 ul (0.18 ml) Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS -- THEY HAVE ALREADY BEEN PRE-DILUTED 10-FOLD
2. Secure the desired number of coated wells in the holder.
3. Dispense 20ul of myoglobin standards, diluted specimens and diluted controls into the appropriate wells.
4. Dispense 200ul of Enzyme Conjugate Reagent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix completely.
6. Incubate at room temperature (20-25°C) for 45 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water drops.
10. Dispense 100ul of TMB Reagent solution into each well. Gently mix for 5 seconds.
11. Incubate at room temperature for 20 minutes.
12. Stop the reaction by adding 100ul of Stop Solution to each well.
13. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
14. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

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