BOSTER

antibody and ELISA experts

Human High Sensitivity C-Reactive Protein (CRP) ELISA Kit

Catalog number: EK7040

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 High Sensitivity C-Reactive Protein (CRP) ELISA Kit

Catalog Number: EK7040

Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 0.005 mg/L

Assay Range: 0.005-0.1 mg/L

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 direct ELISA sandwich kit. Instead of adding samples detection antibody and ABC-HRP separately, The OneStep ELISA kit allows the user to add standards, samples and controls to wells in one step, along with the incubation buffer. After a simple washing step, an enzyme conjugate reagent is added into each well. 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. Microwells coated with CRP MAb	12x8x1
2. CRP Standard: 6 vials (ready to use)	0.7ml
3. CRP Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. Sample Diluent	50 ml
7. 20X Wash concentrate: 1 bottle	25ml

Standard Concentrations and example data

	OD 450 nm	Conc. mg/L
Std 1	0.02	0
Std 2	0.23	0.005
Std 3	0.49	0.01
Std 4	1.01	0.025
Std 5	1.66	0.05
Std 6	2.4	0.1

Materials Required, but Not Provided

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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. 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.
2. This test kit is designed for research use only.
3. 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

1X Wash Buffer: 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

Prior to assay, allow reagents to stand at room temperature (20-25°C). Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder
2. Dilute patient samples and controls 1:100 by adding 5 ul of samples to 495 ul of sample Diluent (STANDARDS ARE READY TO USE).
3. Dispense 10ul of standard, diluted samples and controls into the appropriate wells

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4. Add 100ul of enzyme conjugate to all wells. Tap the holder to remove air bubbles from the liquid and mix well.
5. Incubate for 60 minutes at room temperature (20-25°C).
6. Remove liquid from all wells. Wash wells three times with 300ul of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100ul of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50ul of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:
1. Check CRP 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 IgE standards (vertical axis) against its versus the CRP 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.
4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
5. Patient samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/l.