Human Chromogranin A (CgA) ELISA Kit (Direct EIA)

Catalog number: EK7034

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 Chromogranin A (CgA) ELISA Kit (Direct EIA)

Catalog Number: EK7034

Size: 96wells/kit

Sample Type: Serum and Plasma

Sensitivity: 10 ng/ml

Assay Range: 10-1000 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

Diluted patient serum is added to wells coated with purified antigens. If antibody against target antigen is present, the antibody will bind to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. 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 the color developed is proportional to the amount of IgG specific antibody in the sample.

Kit Components

Description	Quantity
1. Microwells coated with antibody to human CgA.	12 x 8
2. Chromogranin A Standard 1, 1 Vial	0.5 ml
3. Chromogranin A Standards 2-6, 5 Vials (Lyophilized)	Rec. with 0.5 ml DH2O
4. CgA Enzyme Conjugate Concentrate (20X)	0.7 ml
5. CgA Incubation Buffer: 1 Bottle (Ready to use)	12ml
6. CgA Assay Diluent: 1 Bottle	12 ml
7. Sample Diluent: 1 Bottle	6 ml
8. TMB Substrate: 1 Bottle	12 ml
9. Wash Concentrate (20X): 1 Bottle	25 ml
10. Stop Solution: 1 Bottle (Ready to use)	12 ml

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.

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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, 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
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. 20x Enzyme conjugate concentrate: Prepare Chromogranin A enzyme conjugate working solution by 1:20 fold dilution of the enzyme conjugate concentrate with the Assay Diluent. For each strip, it is required to mix 0.95 mL of the assay diluent with 50 uL of the enzyme conjugate concentrate in a clean test tube.
2. 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1X wash buffer at room temperature.
3. Standard: Reconstitute each lyophilized standard with 0.5ml of distilled water. Allow the vials to stand for 10 minutes and then mix thoroughly by gentle inversion to ensure complete reconstitution. Use the standards as soon as possible upon reconstitution. Freeze the remaining standards (except Standard #1), immediately after use. Standards are stable for a maximum of 3 freeze thaw cycles.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder.
2. Add 20ul of CgA standards, controls and patient's sera in each designated microwell.
3. Add 100ul of incubation buffer to each well.

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4. Cover the plate and incubate for 60 minutes at room temperature (20-25°C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 350ul of 1X Wash Buffer. Blot on absorbent paper towels.
5. The concentration of the samples can be read directly from this standard curve. Samples with
7. Cover the plate and incubate for 60 minutes at room temperature (20-25°C) with shaking.
8. Remove liquid from all wells. Wash wells three times with 350ul of 1X Wash Buffer. Blot on absorbent paper towels.
9. Add 100ul of TMB Substrate into each of the wells.
10. Cover the plate, with aluminum foil, and incubate for 15 minutes at room temperature (20-25°C) with shaking.
11. Uncover the plate and add 50ul of Stop Solution into each of the wells. Mix Gently.
12. Read the absorbance at 450 nm within 10 minutes in a microplate reader.

CALCULATION OF RESULTS

The standard curve is constructed as follows:
1. Check CgA 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. Construct a standard curve by plotting the mean absorbance obtained for each reference standard CgA standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the CONCENTRATION (NG/ML) 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.