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Cat#: CN101T (96 Tests)
For Order and Inquiries, please contact
Calbiotech Inc.,
10461 Austin Dr, Spring Valley, CA, 91978
Tel (619) 660-6162, Fax (619) 660-6970,
www.calbiotech.com

CALBIOTECH
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Chromogranin A (CgA) ELISA

Catalog No. : CN101T (96 Tests)

INTENDED USE

The Calbiotech Chromogranin A ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human Chromogranin A levels in human serum or plasma. For research use only.

SUMMARY AND EXPLANATION

Chromogranin A (CgA) is a 49 KD glycoprotein. Elevated levels of CgA are used to differentiate between neuroendocrine and non-neuroendocrine tumors. About 50% of neuroendocrine tumors have elevated concentration of CgA. Serum CgA is most frequently elevated in gastrinoma (100%), pheochromocytoma (89%), carcinoid tumors (80%), nonfunctioning tumors of the endocrine pancreas (69%) and medullary carcinoma of the thyroid (50%). Only 7% of control subjects have elevated CgA and only 2% have extremely elevated serum CgA (>300 ng/ml).

PRINCIPLE OF THE TEST

The CgA is a solid phase direct sandwich ELISA method. The standards, samples and controls are added into the selected wells coated with anti hCgA monoclonal anybody. CgA in the standards, controls and patient's serum binds to anti-CgA Ab on the wells. Unbound protein is washed off by wash buffer. The anti-hCgA-HRP conjugated second antibody is added and then binds to CgA. Unbound HRP conjugate is washed off by wash buffer. Upon the addition of the substrate, the enzyme activities are proportional to the concentration of CgA in the samples. A standard curve is prepared relating color intensity to the concentration of the CgA.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with antibody to human CgA.	12 x 8
2.	Chromogranin A Standards: 8 Vials (Frozen)	0.125 ml
3.	CgA Enzyme Conjugate Concentrate (20X)	0.7 ml
4.	CgA Incubation Buffer: 1 Bottle (Ready to use)	12ml
5.	CgA Assay Diluent: 1 Bottle	12 ml
6.	Sample Diluent: 1 Bottle	6 ml
7.	TMB Substrate: 1 Bottle	12 ml
8.	Wash Concentrate (20X): 1 Bottle	25 ml
9.	Stop Solution: 1 Bottle (Ready to use)	12 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C. Keep Standard <-20° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

4. Do not expose reagents to heat, sun, or strong light.

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, 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
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.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate
6. 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 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.

REAGENTS 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.

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 20 µl of CgA standards, controls and patient's sera in each designated microwell.
3. Add 100 µl of incubation buffer to each well.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26° C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 350 µl of 1X Wash Buffer. Blot on absorbent paper towels.
6. Add 100 µl of the working enzyme conjugate solution to each well (Refer to reagent preparation).
7. Cover the plate and incubate for 60 minutes at room temperature (18-26° C) with shaking.
8. Remove liquid from all wells. Wash wells three times with 350 µl of 1X Wash Buffer. Blot on absorbent paper towels.
9. Add 100 µl of TMB Substrate into each of the wells.
10. Cover the plate, with aluminum foil, and incubate for 15 minutes at room temperature (18-26° C) with shaking.
11. Uncover the plate and add 50 µl 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. To construct the standard curve, plot the RLU (Relative Light Units) for each CgA standard point (vertical axis) versus the 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

Example of a standard Curve

A typical absorbance data and the resulting standard curve from human Chromogranin A EUSA are represented. This curve should not be used in lieu of standard curve run with each assay.

Standard	OD	Conc.ng/mL
Std 1	0.02	0
Std 2	0.20	20
Std 3	0.35	40
Std 4	0.67	75
Std 5	1.21	150
Std 6	1.86	300
Std 7	2.47	600
Std 8	3.04	1200

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:
Normal Range: Less than 40 ng/ml

LIMITATION OF THE PROCEDURE

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