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Chromogranin A (CgA) ELISA Kit

rev 03/17

(Catalog # K4241-100, 100 assays, Store at 4°C)

I. Introduction:

Chromogranin A or parathyroid secretory protein 1 (gene name CHGA) is a member of the granin family of neuroendocrine secretory proteins, It is located in secretory vesicles of neurons and endocrine cells such as islet beta cell secretory granules in pancreas. It strongly inhibits glucose induced insulin release from the pancreas. BioVision's Chromogranin A ELISA kit is a sandwich ELISA assay for the quantitative measurement of human Chromogranin A in serum, plasma and biological fluid. The density of color is proportional to the amount of human Chromogranin A captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Chromogranin A.

III. Specificity:

Human

IV. Sample Type:

Serum, Plasma and Biological fluid

V. Kit Contents:

Components	K4241-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4241-100-1
Chromogranin A Standards (S1 – S6)	6 vials	K4241-100-2.x
CgA Enzyme Conjugate Concentrate (20X)	0.7 ml	K4241-100-3
CgA Incubation Buffer	12 ml	K4241-100-4
CgA Assay Diluent	12 ml	K4241-100-5
Sample Diluent	6 ml	K4241-100-6
TMB Substrate	12 ml	K4241-100-7
Wash Concentrate (20X)	25 ml	K4241-100-8
Stop Solution	12 ml	K4241-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C. Keep Standards <-20°C, -80°C for long term storage.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

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. **Standards:** Reconstitute each lyophilized standard (S2-S6) with 0.5 ml of ddH_2O. Allow vials to stand for 10 minutes and mix thoroughly by gentle inversion to ensure compete reconstitution. Use the standards as soon as possible after reconstitution. Freeze the remaining standards (Except S1) immediately after use. Standards are stable for maximum of 3 freeze-thaw cycles.
4. **Sample Preparation:** Collect blood specimens and separate the serum immediately. 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Place the desired number of coated strips into the holder.
2. Add 20 µl of **CgA standards**, **controls** and **samples** in each designated microwell.

3. Add 100 µl of incubation buffer to each well. Cover the plate and incubate for 60 minutes at room temperature (20-25°C) with shaking.
4. Remove liquid from all wells. Wash wells three times with 350 µl of **1X Wash Buffer**. Blot on absorbent paper towels.
5. Add 100 µl of the **working enzyme conjugate solution** to each well.
6. Cover the plate and incubate for 60 minutes at room temperature (20-25°C) with shaking.
7. Remove liquid from all wells. Wash wells three times with 350 µl of **1X Wash Buffer**. Blot on absorbent paper towels.
8. Add 100 µl of **TMB Substrate** into each of the wells.
9. Cover the plate, with aluminum foil, and incubate for 15 minutes at room temperature (18-26°C) with shaking.
10. Uncover the plate and add 50 µl of **Stop Solution** into each of the wells. Mix Gently.
11. Read the absorbance at 450 nm within 10 minutes in a microplate reader.

X. CALCULATION:

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).** The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Salbutamol concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Standard	Concentration (ng/ml)	OD 450 nm
S1	0	0.029
S2	5	0.08
S3	20	0.17
S4	75	0.52
S5	280	1.46
S6	800	2.94

XI. RELATED PRODUCTS:

- CA19-9 (human) ELISA Kit (Cat. No. K7427-100)
- Cancer Antigen 125 (CA-125) (human) ELISA Kit (Cat. No. K4803-100)
- Cancer Antigen 15-3 (CA15-3) (human) ELISA Kit (Cat. No. K4804-100)
- Prostate Specific Antigen (Free, human) ELISA Kit (Cat. No. K7432-100)