3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Chromogranin A (CgA) ELISA

Catalog Number **SE120032**

TECHNICAL BULLETIN

Product Description

Chromogranin A (CgA) is a 49 kDa 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).

The Chromogranin A ELISA (enzyme-linked immunosorbent assay) Kit is intended for the quantitative determination of human Chromogranin A levels in human serum or plasma. 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 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.

Components

Materials Provided	96 Tests
Microwells coated with antibody to human CgA	12 x 8
Chromogranin A Standards: 8 Vials (Frozen)	0.125 mL
CgA Enzyme Conjugate Concentrate (20x)	0.7 mL
CgA Incubation Buffer: 1 Bottle (ready to use)	12 mL
CgA Assay Diluent: 1 Bottle	12 mL
Sample Diluent: 1 Bottle	6 mL
TMB Substrate: 1 Bottle	12 mL
Wash Concentrate (20x): 1 Bottle	25 mL
Stop Solution: 1 Bottle (Ready to use)	12 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.

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 µL 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.

Storage/Stability

Store the Chromogranin A Standards at –80 °C and rest of the kit at 2–8 °C.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

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.

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 serum samples 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 (see 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.

Results

Calculations

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 the value is checked on every kit.
2. To construct the standard curve, plot the absorbance 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

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

	OD	Concentration (ng/mL)
Standard 1	0.02	0
Standard 2	0.20	20
Standard 3	0.35	40
Standard 4	0.67	75
Standard 5	1.21	150
Standard 6	1.86	300
Standard 7	2.47	600
Standard 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 value may be used as initial guideline ranges only:

Normal Range: <40 ng/mL

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings. and other diagnostic procedures.Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Product Profile

Sensitivity

The sensitivity of the human Chromogranin A ELISA as determined by the 95% confidence limit on 20 duplicate determinations of zero standard is <5 ng/mL.

Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 20 replicate determinations.

Mean CgA Value ng/mL	CV (%)
63.5	4.2
209	3.6

The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays.

Mean CgA Value ng/mL	CV (%)
61.9	6.7
213.3	5.6

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

Number	Dilution	Observed value	Expected Value	Recovery (%)
1	Neat	286	–	–
	1:2	138	143	96
	1:4	75	72	104
	1:8	37.9	36	105
	1:16	19.5	18	108
2	Neat	61.8	–	–
	1:2	32.1	30.9	104
	1:4	15.9	15.5	103
	1:8	7.2	7.7	94

Recovery

Two patient serum samples were spiked with various amounts of human Chromogranin A (1 vol. + 1 vol. mixture) and assayed. The results in the value of ng/mL are as follows:

Number	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery (%)
1	62.8	31	45.2	46.9	96
		93	75.6	77.9	97
		280	152.8	171.4	89
2	289	31	152.2	160	95
		93	176	191	92
		280	288.2	284.5	101

References

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