

Chromogranin-A-RIA

REF KIPERB321



100

Instructions for Use

English



Please read this user manual carefully before use.

Other translations of this Instruction for Use can be downloaded from our website: <https://www.DiaSource-diagnostics.com>



DIAsource ImmunoAssays S.A.

Rue du Bosquet, 2

1348 Louvain-la-Neuve (Belgium)

INSTRUCTIONS FOR USE (IFU) HISTORY

Version: 1.0

Date: DD.MM.YYYY. - This version supersedes all earlier instruction manuals.

Content changes versus previous version:

Version number	Changes vs previous version
1	• Restructured and rephrased for more clarity and alignment with requirements of "In Vitro Diagnostic Medical Devices Regulation" (IVDR) (EU) 2017/746. • In line with IVDR requirements, information/data have been added on sections Analytical Performance Characteristics, Clinical Performance Characteristics, Warnings and Precautions as well as Limitations. • The following sections were added: Important Notice and Symbols. • The Bibliography section was updated.

Chromogranin-A-RIA KIPERB321

This device is a radiometric immunoassay (precipitation) for the quantitative determination of human chromogranin-A (CgA) in human serum.

FOR PROFESSIONAL USE ONLY

MAIN ASSAY FEATURES

Assay principle	radiometric
Sample type	serum
Sample volume	100 µL (10x diluted)
Incubation time	20-24 hours + 30-60 min at 2-8 °C
Results are given in	nmol/L
Linear direct measuring range	2.2 - 70 nmol/L
Number of calibrators	6

DiaSource SERVICES

Info	info@diasource.be	+32 (0) 10 84 99 11
Customer Service	customer.service@diasource.be	+32 (0) 10 84 99 00
Product Managers	products.support@diasource.be	

1. INTENDED PURPOSE

The DiaSource Chromogranin-A-RIA is a non-automated radioimmunoassay (RIA) intended to be used for the quantitative measurement of chromogranin-A (CgA) in human serum by trained professionals in medical laboratories.

The *in vitro* diagnostic medical device is intended to be used for aiding in the diagnosis, for monitoring and for post-operative follow-up of neuroendocrine tumors (NETs).

The measurement of CgA is useful in patients with suspected or confirmed NET.

2. GENERAL INFORMATION

A neuroendocrine tumor is a heterogeneous neoplasm arising from the neuroendocrine cells of the diffuse endocrine system and secreting a bunch of substances (Bevere *et al.*, 2023; Komarnicki *et al.*, 2022; Ciobanu *et al.*, 2021; Regolo *et al.*, 2023; Koffas *et al.*, 2023; Mukherjee *et al.*, 2024). Among them, chromogranin-A (CgA) is a 439 amino acid glycoprotein of the granin family released together with peptide hormones and biogenic amines as part of the neuroendocrine system (Bevere *et al.*, 2023; Komarnicki *et al.*, 2022; Ciobanu *et al.*, 2021; Koffas *et al.*, 2023).

Therefore, it represents a broad-spectrum marker for NETs in both functioning and nonfunctioning tumors showing various sensitivity and specificity (varying between 42-93% and 56-100%, respectively) depending on the tumor type, location and burden (Komarnicki *et al.*, 2022; Koffas *et al.*, 2023). In addition, CgA shows great value in monitoring the progression of the disease and treatment response as well as in predicting recurrence (Komarnicki *et al.*, 2022).

The diagnostic cascade should be initiated once there is any clinical suspicion. Initial work-up includes assessment of peptides hormones such as Chromogranin-A, and, in selected patients with symptoms indicative of a specific endocrine syndrome, the specific marker in question (although this question will not be tackled here). Radiological and functional imaging is then used to complement the diagnostic approach as well as evaluate disease extension (staging), assess the response to therapy and localize the primary site. Ultimately, the gold-standard of the diagnosis remains histopathology (Regolo *et al.*, 2023; Koffas *et al.*, 2023).

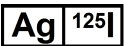
3. TEST PRINCIPLE

The operation principle of radioimmunoassay (RIA) by precipitation is based on the competition between the radioactive and non-radioactive antigen for a fixed number of antibody binding sites. The amount of ¹²⁵I-labelled analyte that binds to the antibody is inversely proportional to the concentration of unlabelled analyte present.

Antibody-bound analyte is separated from the unbound fraction using a Double Antibody (DA) precipitation technique. The radioactivity of the precipitates (corresponding to the antibody-bound ¹²⁵I-labelled analyte fraction) is measured with a gamma counter.

4. MATERIAL PROVIDED

The Chromogranin-A-RIA kit comprises the following major components:

Name	Symbol	Label color	Origin	Description	Reconstitution
Tracer (iodine 125 Ag)		Red		1 vial (12.5 mL) of ¹²⁵ Iodine labeled recombinant human Chromogranin A (56 kBq) in phosphate buffered saline solution with bovine serum albumin, and sodium azide (< 0.1%).	Ready for use.
Antiserum		Blue		1 vial of lyophilized rabbit antiserum to human chromogranin A (amino acids 116-439) in phosphate buffered saline solution with bovine serum albumin.	Add 11 mL distilled water.
DASP		Green		1 vial (52 mL) of Anti-rabbit-Ig coupled to cellulose particles in phosphate buffered saline solution with bovine serum albumin, and sodium azide (< 0.1%).	Ready for use.
Calibrator		Yellow		1 vial of lyophilized calibrator in phosphate buffered saline solution containing recombinant CgA, bovine serum albumin, and sodium azide (< 0.1%).	Reconstitute with distilled water by the volume stated on the vial label.
Control 1		Grey		1 vial of lyophilized recombinant CgA in phosphate buffered saline solution containing bovine serum albumin, and sodium azide (< 0.1%). See exact value on the vial label.	Add 1 mL distilled water.
Control 2		Grey		1 vial of lyophilized recombinant CgA in phosphate buffered saline solution containing bovine serum albumin, and sodium azide (< 0.1%). See exact value on the vial label.	Add 1 mL distilled water.
Assay Buffer		Black		1 vial (50 mL) of phosphate buffered saline solution containing bovine serum albumin, and sodium azide (< 0.1%). To be used for dilution of samples, preparation of working calibrators and for replacement of antiserum in non-specific binding controls.	Ready for use.

5. MATERIAL NOT PROVIDED

The following material is required but not provided in the kit:

- Vortex mixer
- Aspiration system
- Any gamma counter capable of measuring iodine-125 radiation may be used (with a minimal yield of 70%)
- Distilled water
- Disposable test tubes of polystyrene: 11-13x55mm
- Centrifuge, refrigerated, giving a minimum of 1700 x g
- Pipettes for accurate delivery of different volumes (the use of accurate pipettes with disposable plastic tips is recommended)

6. SPECIMEN COLLECTION, HANDLING AND PREPARATION

Only the following specimen types must be used: human serum.

It is the responsibility of the operator to ensure that the correct specimen types are used.

Observe the following recommendations for handling, processing and storing of samples:

- Specimen collection is performed according to Good Clinical Laboratory Practice (GCLP).
- The human blood collection is done by venipuncture in red tubes without additives or in tubes containing a clot activator.
- Allow serum samples to clot completely before centrifugation.
- The human serum should be promptly separated from blood cells, preferably in a refrigerated centrifuge.
- Repeated freezing and thawing must be avoided.
- If a specimen is expected or known to have a concentration above the most concentrated calibrator, it must be diluted with the assay buffer so that its concentration falls within the measuring interval.

These human serum sample stabilities are given as an indication (Thirkettle et al., 2023):

Temperature	Time
Ambient (20 - 25°C)	24 hours
Refrigerated (2 - 8°C)	3 days
Frozen (preferred) (-20°C)	7 days

7. STORAGE CONDITIONS AND SHELF LIFE OF REAGENTS

Before opening or reconstitution, all kit components are stable until the expiration date indicated on the label if kept **at 2 to 8°C**, based on the assessment of the measurand drift following the guidelines of the CLSI document EP25.

After first use, the components are stable until the **kit expiration date** (indicated on the kit label), based on the assessment of the measurand drift following the guidelines of the CLSI document EP25, if stored and handled as indicated in the following **Table**:

Name	Storage temperature after first opening	Handling directions
Tracer (iodine 125 Ag)	2 - 8 °C	• Keep in the original, well-closed vial. • Only one freeze-thaw cycle allowed.
Antiserum	2 - 8 °C	• Keep in the original, well-closed vial.
DASP	2 - 8 °C	• Keep in the original, well-closed vial. • Stir continuously during pipetting.
Calibrator	-20 °C or lower.	• Freeze immediately after use. • Only one freeze-thaw cycle allowed. • Keep in the original, well-closed vial.
Control 1	-20 °C or lower.	• Freeze immediately after use. • Only one freeze-thaw cycle allowed. • Keep in the original, well-closed vial.
Control 2	-20 °C or lower.	• Freeze immediately after use. • Only one freeze-thaw cycle allowed. • Keep in the original, well-closed vial.
Assay Buffer	2 - 8 °C	• Keep in the original, well-closed vial.

8. TEST PROCEDURE

INTENDED USERS

The device shall be used only by trained professionals in medical laboratories equipped with radioactive safety procedures according to local regulations.

QUALITY CONSIDERATIONS

- Do not use the kit or components beyond expiry date.
- Do not mix components from different kit lots.
- Bring all the reagents to room temperature (18°C to 25°C) for 15 minutes prior to use.
- Thoroughly mix all reagents and samples by gentle agitation or swirling.
- In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.
- High precision pipettes or automated pipetting equipment will improve assay precision.
- Respect the incubation times.
- Prepare a calibration curve for each run, do not use data from previous runs.
- Patient samples with a concentration above the measuring range are to be rated as "> most concentrated calibrator". The result must not be extrapolated. The patient sample in question should be diluted and retested.
- The water used for reconstitution of the lyophilized reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the contents in the vials by gentle inversion and avoid foaming.
- Patient samples with a concentration below the LoQ are to be rated as "< LoQ". The result must not be extrapolated.

REAGENTS PREPARATION

- **Tracer (iodine 125 Ag)**
Ready for use.
- **Antiserum**
Add 11 mL distilled water.
- **DASP**
Ready for use.
- **Calibrator**
Reconstitute with distilled water by the volume stated on the vial label.
- **Control 1**
Add 1 mL distilled water.
- **Control 2**
Add 1 mL distilled water.
- **Assay Buffer**
Ready for use.

PROCEDURE

1. Bring the reagents to room temperature prior to use.
2. Reconstitute the lyophilized reagents according to the instructions.
3. Label tubes in duplicate for total counts (TC), non-specific binding (NSB), calibrators 0-6, controls and samples.
4. Prepare the Chromogranin A working calibrators by dilution of the 14 nmol/L **unique calibrator** with the Assay Buffer according to the following (store at -20°C or lower if reused):

- a/ **Calibrator 6** = 0.40 mL calibrator 14 nmol/L + 0.40 mL Assay Buffer = 7.00 nmol/L.
b/ **Calibrator 5** = 0.40 mL calibrator 7.00 nmol/L + 0.40 mL Assay Buffer = 3.50 nmol/L.
c/ **Calibrator 4** = 0.40 mL calibrator 3.50 nmol/L + 0.40 mL Assay Buffer = 1.75 nmol/L.
d/ **Calibrator 3** = 0.40 mL calibrator 1.75 nmol/L + 0.40 mL Assay Buffer = 0.88 nmol/L.
e/ **Calibrator 2** = 0.40 mL calibrator 0.88 nmol/L + 0.40 mL Assay Buffer = 0.44 nmol/L.
f/ **Calibrator 1** = 0.40 mL calibrator 0.44 nmol/L + 0.40 mL Assay Buffer = 0.22 nmol/L.
g/ **Calibrator 0** = Assay Buffer = 0 nmol/L.

5. Dilute the samples 1:10 with Assay Buffer (e.g. 50 µL sample and 450 µL Assay Buffer). Vortex-mix carefully.

6. Dispense **100 µL** of calibrators (0.00-7.00 nmol/L), diluted samples and controls in their respective tubes.

7. Dispense **200 µL** of Assay Buffer into NSB-tubes.

8. Add **100 µL** of tracer (¹²⁵I-Chromogranin A) into all tubes. The TC-tubes are capped and kept aside until step 15.

9. Add **100 µL** of antiserum into all tubes except the NSB and TC tubes

10. Vortex-mix each tube separately and incubate for **20-24 hours** at **2-8 °C**.

11. Add **500 µL** of **well-mixed** Double Antibody Solid Phase into all tubes except the TC-tubes. **This reagent must be stirred continuously with a magnetic stirrer during pipetting.**

12. Vortex-mix **carefully** each tube separately and incubate for **30-60 min** at **2-8 °C**.

13. Centrifugate for **15 minutes** at minimum **1700 x g** at **4°C**.

14. Aspirate the supernatant **immediately** after centrifugation.

15. Count all tubes, including the TC-tubes, in a gamma counter for **1 minute**.

SUMMARY OF THE PROTOCOL

STEPS	TOTAL COUNTS (μL)	NSB (μL)	CALIBRATOR 0 (μL)	CALIBRATORS 1-6 / CONTROLS (μL)	SAMPLES (μL)
Assay Buffer	-	200 μL	100 μL	-	-
Calibrators/Controls	-	-	-	100 μL	-
Samples 10x diluted	-	-	-	-	100 μL
Tracer	100 μL				
Antiserum	-	-	100 μL	100 μL	100 μL
Incubation	Vortex-mix and incubate for 20-24 hours at 2-8 °C.				
Double Antibody Solid Phase	-	500 μL			
Incubation	Vortex-mix and incubate for 30-60 minutes at 2-8 °C.				
Separation	Centrifugate 15 min (1700 g at 4 °C). Aspirate the supernatant.				
Counting	Count tubes for 1 minute.				

QUALITY CONTROL PROCEDURES

Good Clinical Laboratory Practices (GCLPs) require that control samples are measured regularly to ensure the accuracy and precision of the assay. It is recommended to measure controls with each batch of measurements. These samples must be processed as indicated in the test procedure. It is recommended that the results are analyzed by using appropriate statistical methods. If the results obtained for controls are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.

If desirable, each laboratory can make their own pools of control samples, which should be kept frozen in aliquots. Do not freeze-thaw more than once.

Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Clinical Laboratory Practices.

CALCULATION OF RESULTS

1. Calculate the mean count rate (cpm) of the non-specific binding tubes (NSB) and subtract it from the mean count rate (cpm) of the calibrator, the sample and control tubes (B) as well as total count (TC).
2. Calculate the percent $(B - B_{NSB}) / (T - B_{NSB})$ for calibrators, samples, and controls.
3. A calibration curve is generated by plotting the percent $(B - B_{NSB}) / (T - B_{NSB})$ (ordinate) against the concentration of the calibrators (abscissa).
4. Interpolate the analyte concentrations in the samples and controls from the generated calibration curve. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4 parameter logistic function curve fitting is recommended.
5. Multiply sample test result 10 times.

STANDARD CURVE EXAMPLE

The following data are for illustration only and should never be used instead of the real time calibration curve.

		cpm	$(B - B_{NSB}) / (T - B_{NSB}) \times 100 (\%)$
Total count		24658.0	/
Non-specific binding		617.7	/
Calibrators (nmol/L)	0.0	13207.0	52.37
	0.22	11819.7	46.60
	0.44	11354.0	44.66
	0.88	9950.0	38.82
	1.75	8113.0	31.18
	3.50	5756.3	21.37
	7.00	4069.0	14.36

9. WARNINGS AND PRECAUTIONS

	Description
	Consult Instructions for Use (IFU).
	This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals. All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the laboratory. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes. Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
	All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
	This product contains concentrations of azide below the hazardous level which with repeated contact with lead and copper commonly found in plumbing drains may result in the build up of shock sensitive compounds. Sodium azide forms explosive compounds with heavy metals.
	Do not smoke, drink, eat or apply cosmetics in the working area.
	Observe the generally acknowledged safety precautions and laboratory techniques when handling reagents and patient samples.
	For more information, see Material Safety Data Sheet (MSDS) .
	Do not use after expiration date specified on the label (format YYYY-MM-DD).
	Reagents from different kit lots should not be mixed .
	The concentration of the analyte in a given patient sample, determined with assays from different manufacturers , can vary due to differences in assay methods, calibration, and reagent specificity . Values measured with assays from different manufacturers cannot be interchangeably used .

10. LIMITATIONS

- This product is **not** intended to be used as a **stand-alone diagnostic test**. The results of this test should only be interpreted in conjunction with other diagnostic measures, symptoms and clinical assessment.
- The kit **components** are stable until expiry date if kept in indicated storage conditions and may be **reused only once**.
Prepare a **calibration curve for each run**, do not use data from previous runs.
Alterations in physical appearance of kit reagents may indicate instability or deterioration which may affect the performances. In this case, the kit reagents should **no longer be reused**.
- **Heterophilic antibodies** in human blood can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.
Patients routinely **exposed to animals or animal serum products** can be prone to this interference and **anomalous values may be observed in case of the presence of heterophilic antibodies**. Carefully evaluate the results of patients suspected of having these antibodies.
If results are not consistent with other clinical observations, additional information should be required before diagnosis.
- In the context of **patient follow-up**, CgA results should be interpreted with **caution** and in conjunction with the patient's clinical status. Observed variability in CgA levels may be influenced in part by the analytical **reproducibility of the assay**.

11. ANALYTICAL PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Assay results obtained in individual laboratories may vary from data presented.

ACCURACY OF MEASUREMENT

TRUENESS OF MEASUREMENT

In the absence of a recognized reference measurement procedure or certified reference material, trueness was assessed through **agreement with a comparator method**.

A method comparison study was performed between the DiaSource Chromogranin-A-RIA assay and another commercially available immunoassay measuring CgA using **30 native human serum samples**. Samples were classified as positive or negative according to the respective assay cut-off values. The **Positive Percent Agreement (PPA) was 100%** (95% CI: [85.7–100]), and the **Negative Percent Agreement (NPA) was 85.7%** (95% CI: [48.7–97.4]).

PRECISION OF MEASUREMENT

3 human serum samples have been measured by **3 operators** on **5 days**, with **2 runs per day**, in **3 replicates** using **4 reagent lots**, according to the guidelines of the CLSI document EP05-A3. Results from this study for selected samples are summarized in the following **Table**:

Lot	Sample	Mean (nmol/L)	Repeatability (%CV)	Within-laboratory (%CV)	Reproducibility (%CV)
A	1	3.69	12.8	16.0	24.9
	2	10.87	6.1	13.4	17.1
	3	33.09	9.2	15.1	16.3
B	1	3.65	13.8	16.8	24.0
	2	10.63	5.6	13.0	17.5
	3	32.71	5.3	12.2	14.7
C	1	3.93	7.7	13.1	21.4
	2	12.17	5.0	14.1	26.3
	3	31.06	5.1	13.9	28.3
D	1	3.95	6.8	12.3	23.5
	2	11.85	4.9	12.4	25.4
	3	31.29	4.7	15.0	30.3

ANALYTICAL SENSITIVITY

LIMIT OF BLANK (LoB)

The Limit of Blank (LoB) for the Chromogranin-A-RIA kit is 0.26 nmol/L, determined consistent with the guidelines of the CLSI document EP17-A2. **Four blank samples** (assay buffer) have been measured by **1 operator** in **5 replicates** per run on **2 kit lots** for **3 days**.

LoB	0.26 nmol/L
-----	-------------

LIMIT OF DETECTION (LoD)

The Limit of Detection (LoD) for the Chromogranin-A-RIA kit is 0.71 nmol/L, determined consistent with the guidelines of the CLSI document EP17-A2 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on **360 determinations**, with **4 blank** and **10 low level replicates** (i.e. **4 blank samples** in **5 replicates** per run and **10 human serum samples** with low Chromogranin-A level in **4 replicates** per run, measured by **1 operator** on **2 kit lots** for **3 days**); and an LoB of 0.26 nmol/L.

LoD	0.71 nmol/L
-----	-------------

LIMIT OF QUANTITATION (LoQ)

The Limit of Quantitation (LoQ) for the Chromogranin-A-RIA kit is 1.01 nmol/L, determined consistent with the guidelines of the CLSI document EP17, based on **240 determinations** (i.e. **10 human serum samples** with low Chromogranin-A level, measured by **1 operator** in **4 replicates** per run on **2 kit lots** for **3 days**) ; the LoQ was determined as the lowest Chromogranin-A concentration that can be reproducibly measured with an **intermediate precision CV goal of 25%**.

LoQ	1.01 nmol/L
-----	-------------

ANALYTICAL SPECIFICITY

INTERFERING SUBSTANCES

Potentially interfering substances were determined according to the guidelines of the CLSI documents EP07 and EP37. Substances tested for their potential to interfere with assay performance (see **Table**) were added to **3 different human serum samples** with Chromogranin-A concentration between 2.25 and 41.69 nmol/L. They were found to **not interfere at the concentrations expected in the intended patient population** and **indicated** in the following **Table**:

Interfering substance	Tested concentrations
Bilirubin, conjugated	40 mg/dL
Bilirubin, unconjugated	40 mg/dL
Glucose	1000 mg/dL
Rheumatoid Factor	100 IU/mL
Total cholesterol	400 mg/dL
Triglycerides	1500 mg/dL
Hemoglobin	250 - 1000 mg/dL
Acetylsalicylic acid	2.04 mg/dL
Paracetamol	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Omeprazole	0.84 mg/dL
Ranitidine	1.05 mg/dL
Hydrocortisone	30 µg/dL

CROSS-REACTIVE SUBSTANCES

Potentially cross-reactive substances (i.e. interferents structurally similar to the antigen of interest) were tested according to the guidelines of the CLSI document EP07. Substances assayed for their potential to interfere with assay performance (see **Table**) were added to **3 different human serum samples** with Chromogranin-A concentration between 2.86 and 35.42 nmol/L. They were found to **not cross-react at the concentrations indicated** in the following **Table**:

Interfering substance	Tested concentrations
Chromogranin B (CgB)	12.76 nmol/L
Chromogranin C (CgC)	12.76 nmol/L

METROLOGICAL TRACEABILITY OF CALIBRATOR AND CONTROL MATERIAL VALUES

The Chromogranin-A-RIA kit is calibrated against a commercially available recombinant human chromogranin-A protein. This calibration is verified on a regular basis by regression analysis and comparison to theoretically expected results using 1 reagent lot.

The chromogranin-A (CgA) measurement results are given in nmol/L.

The uncertainty of assigned values for the Chromogranin-A-RIA kit calibrators is %ru Cal = 15.7%..

MEASURING RANGE OF THE ASSAY

LINEARITY

The kit was tested for linearity from **2.2 to 70 nmol/L** and has been demonstrated to **be linear** with a **maximum allowable nonlinearity of 15%**, according to the guidelines of the CLSI document EP06.

DEFINITION OF ASSAY CUT-OFF

The assay cut-off was determined according to literature search to be 4.7 nmol/L in Chromogranin-A concentration (Belli *et al.*, 2009; Molina *et al.*, 2011; Stridsberg *et al.*, 2003; Hoej *et al.*, 2014).

12. CLINICAL PERFORMANCE CHARACTERISTICS

REFERENCE RANGE

A reference range was established based on **human serum samples from 133 presumed healthy individuals**.

The characteristics of the reference interval is given in the following **Table**:

Identification	N	Mean (nmol/L)	Median (nmol/L)	SD (nmol/L)	95% upper reference limit (nmol/L)	90% CI (nmol/L)
Presumed healthy individuals	133	2.820	2.600	0.878	4.46	3.90 - 5.00

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

INTERPRETATION OF RESULTS

Analyte values need to be evaluated in combination with clinical symptoms and/or other laboratory parameters. Values higher than the 95% upper reference limit, as stated in the Table above, should be considered pathological with respect to the intended use and under consideration of the limitations.

Note: It is recommended that each laboratory establishes its own reference range based on representative patient collectives and/or test the validity of the manufacturer's commercial test kit data.

DIAGNOSTIC SPECIFICITY AND SENSITIVITY

Several studies support the use of Chromogranin A (CgA) testing with the Chromogranin-A-RIA kit for the diagnosis, monitoring, and post-operative follow-up of neuroendocrine tumors (NETs). In particular, the study by Belli et al. (Belli et al., 2009) reported high clinical performance, notably high specificity (100%) and sensitivity (92.3%) across different populations, including healthy subjects and patients with localized or metastatic gastroenteropancreatic NETs (GEP-NETs). This study highlighted the utility of CgA measurement for both the diagnosis and follow-up of GEP-NETs. However, Molina et al. (Molina et al., 2011), in a comparative study evaluating three different immunoassays for CgA measurement, reported lower specificity (88%) and sensitivity (65.2%) for the Chromogranin-A-RIA kit, indicating a more moderate diagnostic performance for diagnosis and screening purposes.

As a good correlation between plasma and serum samples was demonstrated by Glinicki et al. (Glinicki et al., 2015), additional clinical studies using the Chromogranin-A-RIA kit with plasma samples were also considered. Studies by Stridsberg et al. (Stridsberg et al., 2003) and Hoej et al. (Hoej et al., 2014) further demonstrated that Chromogranin A is an important tumor marker for NETs. The first study reported a diagnostic sensitivity of 93%, specificity of 88%, positive predictive value of 93%, and negative predictive value of 88%. The second study reported a sensitivity of 79%; however, this study included only NET patients and did not include a control group.

Overall, the average of these results is consistent with the CgA meta-analysis performed by Yang et al. (Yang et al., 2015). Regarding the RIA-based studies included in this analysis, CgA measurement in NET patients and healthy controls demonstrated a pooled sensitivity and specificity of 0.74 and 0.95, respectively.

13. IMPORTANT NOTICE

Any serious incident that has occurred in relation to the device shall be reported to DIAsource ImmunoAssays S.A. and to the Competent Authority of the country in which the user and/or the patient is established.

The Summary of Safety and Performance is available upon request.

14. BIBLIOGRAPHY

Belli, Susana Haydée, Adriana Oneto, Claudio Aranda, et al. 2009. "Chromogranin A as a Biochemical Marker for the Management of Neuroendocrine Tumors: A Multicenter Study Developed in Argentina". *Acta Gastroenterol Latinoam* 39 (3).

Bevere, Michele, Francesca Masetto, Maria Elena Carazzolo, et al. 2023. "An Overview of Circulating Biomarkers in Neuroendocrine Neoplasms: A Clinical Guide." *Diagnostics* 13 (17): 2820. <https://doi.org/10.3390/diagnostics13172820>.

Ciobanu, Oana, Sorina Martin, and Simona Fica. 2021. "Perspectives on the Diagnostic, Predictive and Prognostic Markers of Neuroendocrine Neoplasms (Review)." *Experimental and Therapeutic Medicine* 22 (6): 1479. <https://doi.org/10.3892/etm.2021.10914>.

Glinicki, Piotr, Wojciech Jeske, Renata Kapuścińska, and Wojciech Zgliczyński. 2015. "Comparison of Chromogranin A (CgA) Levels in Serum and Plasma (EDTA2K) and the Respective Reference Ranges in Healthy Males." *Endokrynologia Polska* 66 (1): 53–56. <https://doi.org/10.5603/EP.2015.0009>.

Hoej, Lise Brehm, Tina Parkner, Cindy Soendersoe Knudsen, and Henning Grønbaek. 2014. "A Comparison of Reex Chromogranin A Assays in Patients with Neuroendocrine Tumours." *Journal of Gastrointestinal and Liver Diseases* 23 (4): 419–24. <https://doi.org/10.15403/jgld.2014.1121.234.3ca>.

Koffas, Apostolos, Alexandros Giakoustidis, Apostolis Papaefthymiou, et al. 2023. "Diagnostic Work-up and Advancement in the Diagnosis of Gastroenteropancreatic Neuroendocrine Neoplasms." *Frontiers in Surgery* 10 (March): 1064145. <https://doi.org/10.3389/fsurg.2023.1064145>.

Komarnicki, Paweł, Jan Musiałkiewicz, Alicja Stańska, et al. 2022. "Circulating Neuroendocrine Tumor Biomarkers: Past, Present and Future." *Journal of Clinical Medicine* 11 (19): 5542. <https://doi.org/10.3390/jcm11195542>.

Molina, Rafael, Elias Alvarez, Angeles Aniel-Quiroga, et al. 2011. "Evaluation of Chromogranin A Determined by Three Different Procedures in Patients with Benign Diseases, Neuroendocrine Tumors and Other Malignancies." *Tumor Biology* 32 (1): 13–22. <https://doi.org/10.1007/s13277-010-0085-x>.

Mukherjee, Sreetama, Sunita Vagha, and Manish Mukherjee. 2024. "Various Markers of Neuroendocrine Tumor: A Narrative Review." *Cureus, ahead of print*, August 22. <https://doi.org/10.7759/cureus.67493>.

Regolo, Matteo, Nicolas Cardaci, Clara Salmeri, et al. 2023. "Pancreatic Neuroendocrine Tumor (Pan-NET) Presented by Abdominal Pain: A Case Report and Literature Review." *Journal of Clinical Medicine* 12 (20): 6617. <https://doi.org/10.3390/jcm12206617>.

Stridsberg, M., B. Eriksson, K. Oberg, and Et Janson. 2003. "A Comparison between Three Commercial Kits for Chromogranin A Measurements." *Journal of Endocrinology* 177 (2): 337–41. <https://doi.org/10.1677/joe.0.1770337>.

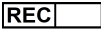
Thirkettle, Sally, Patrycja Blaszczyk, Rachel Evans, et al. 2023. "Stability Assessment of Serum Tumour Markers: Calcitonin, Chromogranin A, Thyroglobulin and Anti-Thyroglobulin Antibodies." *Annals of Clinical Biochemistry* 60 (3): 212–16. <https://doi.org/10.1177/00045632231160371>.

Yang, Xin, Yuan Yang, Zhilu Li, et al. 2015. "Diagnostic Value of Circulating Chromogranin A for Neuroendocrine Tumors: A Systematic Review and Meta-Analysis." *PLOS ONE* 10 (4): e0124884. <https://doi.org/10.1371/journal.pone.0124884>.

15. SYMBOLS

Symbols used in Instructions for Use and Product Labelling of DiaSource Chromogranin-A-RIA kit are summarized in the following Table, except for symbols of the kits components that are present in the section "4. MATERIAL PROVIDED":

Symbol	Title	Description
	Manufacturer	Indicates the manufacturer of the medical device. This symbol is accompanied by the name and address of the manufacturer adjacent to the symbol.
	Date of manufacture	Indicates the date on which the medical device was manufactured. The date is expressed in the form YYYY-MM-DD.
	Use-by date	Indicates the date after which the medical device must no longer be used. The date is expressed in the form YYYY-MM-DD.
	Lot Number	Indicates the manufacturer's batch number.
	Catalog number	Indicates the manufacturer's catalog number.
	Temperature limit	Indicates the minimum and maximum temperatures to which the medical device can be safely exposed, e.g. 2°C to 8°C.
	Biological risks	Indicates that there are potential biohazards associated with the medical device.
	Do not re-use	Indicates a component or kit intended for single-use.
	Consult Instructions for Use	Indicates that it is necessary to consult the Instructions for Use.

Symbol	Title	Description
	<i>In vitro</i> diagnostic medical device	Indicates a medical device that is intended to be used as an <i>in vitro</i> diagnostic medical device.
	Contains sufficient for	Indicates the total number of tests that can be performed with the medical device, e.g. 96.
	Not for self-testing	Indicates that the product cannot be used by a patient himself but must be used in a laboratory.
	Translation	Indicates that the original information of the medical device has been translated in addition to or instead of the original information.
	CE Marking	CE Conformity Marking according to Regulation (EU) 2017/746 on <i>In Vitro</i> Diagnostics Medical Devices, with registration number of Notified Body.
	Unique Device Identification	Indicates a carrier which contains information about a unique device identification.
	Human blood or plasma derived products	Indicates an <i>in vitro</i> diagnostic medical device that contains or incorporates products derived from human blood or plasma.
	Biological products from animal origin	Indicates an <i>in vitro</i> diagnostic medical device that contains biological tissues or cells, or derived elements from animal origin.
	Human biological product	Indicates an <i>in vitro</i> diagnostic medical device that contains biological tissues or cells, or derived elements from human origin.
	Lyophilized product	Indicates that the product is lyophilized.
	Reconstitution	Indicates that the product must be reconstituted in the volume indicated in the box on the right.
	Radioactive	Indicates the presence of a radioactive substance.
	Irritant, sensitising, harmful	These are substances which, absorbed through the skin, may give rise to such a reaction of hypersensitisation (hypersensitivity) that subsequent exposure to the substance or preparation will cause characteristic adverse effects.