thermoscientific

B·R·A·H·M·S CgA II KRYPTOR

Instructions for Use

B·R·A·H·M·S CgA II KRYPTOR

IVD
FOR PROFESSIONAL USE ONLY

The manufacture and/or use of this product is covered by one or more of the following patents:
www.brahms.de/patents

Date: 01.03.2023

This version supersedes all earlier versions.
Changes versus the previous version

- Restructured and rephrased for more clarity and alignment with requirements of European Union “In Vitro Diagnostic Medical Devices Regulation” (IVDR)
- In line with IVDR requirements information/data has been added on: specimen handling, processing, storage, trueness, linear measuring range
- LoD and LoQ updated
- Additional data on medications added in section “Analytical Specificity”
- Important notice added on incidence reporting and safety/performance summary on EUDAMED
- Revision 5.1: Additional cut-off added based on change of CgA values over time in section “Diagnostic Sensitivity & Specificity”
- Revision 5.2: Symbols updated throughout document

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CE 0197

■ Intended Purpose / Intended Use

The B·R·A·H·M·S CgA II KRYPTOR test is an automated immunofluorescent assay for the quantitative determination of chromogranin A (CgA) in human serum or plasma (EDTA).

The B·R·A·H·M·S CgA II KRYPTOR test is indicated as an aid to be used in conjunction with clinical evaluation for follow-up and monitoring of patients with neuroendocrine tumors (NETs) and for therapy monitoring of patients with prostate cancer. The test is further indicated as an aid to be used in conjunction with clinical evaluation for the diagnosis of patients with suspected pheochromocytomas.

■ Instruments

B·R·A·H·M·S CgA II KRYPTOR may only be used together with the following B·R·A·H·M·S KRYPTOR instruments:
☑ B·R·A·H·M·S KRYPTOR compact PLUS

☑ B·R·A·H·M·S KRYPTOR GOLD
Prior to use, ensure that all requirements for the safe use of the instrument are fulfilled by following the instructions defined in the user manual of the respective instrument.

Availability of instruments is dependent on registration status in the country.

■ Introduction

Chromogranin A (CgA) is an acidic, hydrophilic protein of 49 kDa present in chromaffin granules of the neuroendocrine cells and is a member of the granin family.

Neuroendocrine tumors (NETs) originate from neuroendocrine cells found in neuronal and endocrine tissues throughout the body. The most common sites of NETs are the lung, stomach, appendix, cecum, duodenum, pancreas, jejunum/ileum, colon and rectum [1]. NETs arising from the gastrointestinal (GI) tract are collectively known as gastroenteropancreatic neuroendocrine tumors (GEP-NETs) [2]. Follow up and monitoring investigations of NETs are based on tumor markers together with other diagnostic measures and clinical signs [3, 4]. Besides its use as immunohistological marker, CgA has been well recognized as a general broad-spectrum serum and plasma marker in NETs [5-7].

Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer with neuroendocrine differentiation of prostatic epithelial neoplasms, which express neuroendocrine markers such as CgA [8]. Together with other clinical signs, elevated CgA levels can indicate neuroendocrine differentiation in prostate cancer [9].

Pheochromocytoma (PCC) are rare neuroendocrine tumors arising from chromaffin cells of the adrenal medulla. Although most PCC are benign, hypersecretion of catecholamines and methanephrines from PCC is associated with high morbidity and mortality as it can lead to hypertension, cardiovascular disease and death. Approximately 10% of PCC are malignant with distant metastases [10]. In clinical studies, it was shown that CgA can supplement standard biochemical markers such as catecholamines and its metabolites to detect or exclude pheochromocytoma [11, 12].

Important Notes (e.g. limitations of the method, contraindications, etc.):

This product is not intended to be used as a stand-alone diagnostic test. The result of this test should only be interpreted in conjunction with clinical signs, symptoms and other diagnostic measures.

High levels of CgA could also be found in cases of benign diseases (such as gastro-intestinal disorders, kidney failure and cardiovascular disorders) [13].

Chromogranin A values may rise during treatment with proton pump inhibitors (PPI). It is recommended to stop PPI treatment for at least two weeks before determination of CgA [13].

Patient sample may contain heterophilic antibodies that could cause false high/low results. To address this, non-specific antibodies have been added in excess in the conjugate(s).

■ Contents

Kit

B·R·A·H·M·S CgA II KRYPTOR CONT

REF 839050N Σ 50 2 °C 8 °C see label for expiry date

Name	Quantity	Quality	Description
XL-conjugate	VIAL 1	ready for use	Anti-CgA monoclonal antibody conjugated with Alexa Fluor©647, buffer, bovine albumin, bovine immunoglobulins, murine immunoglobulins, potassium fluoride
K- conjugate	VIAL 1	LYOPH	Anti-CgA monoclonal antibody conjugated with cryptate, buffer, bovine albumin, bovine immunoglobulins, murine immunoglobulins, trehalose, mannitol
Diluent	VIAL 1	ready for use	Human serum, preservative, EDTA
Bar code card	1	ready for use	See the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual. The bar code card contains all information needed for the registration of a new reagent lot.

Calibrator and Controls

B·R·A·H·M·S CgA II KRYPTOR CAL

CAL

Not supplied with the kit.

REF 083991N 2 °C 8 °C see label for expiry date

Intended Purpose/Intended Use: To readjust the standard curve memorized in B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD

Name	Quantity	Quality	Description
B·R·A·H·M·S CgA II KRYPTOR CAL	VIALS 6	LYOPH	Recombinant Chromogranin A in horse serum
Bar code card	1	ready for use	See the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual. The bar code card contains information related to the calibrator lot including its concentration.

B·R·A·H·M·S CgA II KRYPTOR QC

CONTROL

Not supplied with the kit.

REF 083992N 2 °C 8 °C see label for expiry date

Intended Purpose/Intended Use: Quality control on board of B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD for the assay.

Name	Quantity	Quality	Description
B·R·A·H·M·S CgA II KRYPTOR CONTROL 1	VIALS 3	LYOPH	Recombinant Chromogranin A in horse serum
B·R·A·H·M·S CgA II KRYPTOR CONTROL 2	VIALS 3	LYOPH	Recombinant Chromogranin A in horse serum
Bar code card	1	ready for use	See the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual. The bar code card contains information related to the control lot, particularly the target concentrations, the standard deviations obtained and the concentration acceptance ranges. This information is visible on the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD monitor screen in the quality control section.
Bar code stick on labels	32 for each control	ready for use	See the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual. The bar code stick-on labels are used for identifying the controls when assayed on B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD.

Other Requisites

B·R·A·H·M·S KRYPTOR compact Consumables

Not supplied with the kit.

Name	REF
B·R·A·H·M·S KRYPTOR BUFFER	89970
B·R·A·H·M·S KRYPTOR compact SOLUTION 1	89981
B·R·A·H·M·S KRYPTOR compact SOLUTION 2	89982
B·R·A·H·M·S KRYPTOR compact SOLUTION 3	89983
B·R·A·H·M·S KRYPTOR compact SOLUTION 4	89984
B·R·A·H·M·S KRYPTOR compact DILCUP	89985
B·R·A·H·M·S KRYPTOR compact REACT	89986

The operation and maintenance of the B·R·A·H·M·S KRYPTOR compact PLUS are described in the related User Manual.

B·R·A·H·M·S KRYPTOR GOLD Consumables

Not supplied with the kit.

Name	REF
B·R·A·H·M·S KRYPTOR BUFFER	89970
B·R·A·H·M·S Solution 1 KRYPTOR GOLD	89991
B·R·A·H·M·S Solution 2 KRYPTOR GOLD	89992
B·R·A·H·M·S Solution 3 KRYPTOR GOLD	89993
B·R·A·H·M·S Solution 4 KRYPTOR GOLD	89994
B·R·A·H·M·S Dilution Plates KRYPTOR GOLD	89995
B·R·A·H·M·S Reaction Plates KRYPTOR GOLD	89996

The operation and maintenance of the B·R·A·H·M·S KRYPTOR GOLD are described in the related User Manual.

Additionally required

Not supplied with the kit.

- Distilled water
- Pipettes
- Tips
- Sample tubes or micro cups (pediatric tubes).

■ Specimen Collection and Preparation

Only the following specimen types must be used: serum, EDTA plasma.

The instrument is not capable of verifying the specimen type. 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 blood samples

Collect all blood samples observing routine precautions for venipuncture.

- Serum should be collected using standard sampling tubes or tubes containing separating gel.
- Allow serum samples to clot completely before centrifugation.
- For centrifugation specifications, please follow the recommendations of the manufacturer of the blood collection tube.
- Keep tubes stoppered at all times.
- Physically separate serum/plasma from blood cells as soon as possible.
- After centrifugation, the separated serum/plasma sample should be stored tightly stoppered at room temperature (18–25°C) for no longer than 48 hours.
- After centrifugation the separated samples can also be refrigerated (2 - 8 °C) for no longer than 48 hours.
- If the assay will not be completed within 48 hours or for shipment of samples, samples shall be aliquoted and stored frozen at -20°C or lower.
- A frozen sample can be stored for up to 1 month at -20°C.
- The analyte is stable up to 4 freeze-thaw cycles.

Observe the following recommendations when preparing the specimens

- Ensure that residual fibrin and cellular matter have been removed before analysis.
- Sample collection systems from various manufacturers may contain different materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
- Each laboratory should verify the acceptability of its blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and from lot-to-lot.
- Centrifuge samples containing precipitates before running the assay.
- Place the sample in a suitable tube (11–17 mm diameter) for use on KRYPTOR compact PLUS/ KRYPTOR GOLD. This may be the primary tube.
- The sample tube must contain a minimum volume which will vary depending on the tube diameter. A 13 mm diameter tube will require an additional 150 µL of the sample as dead volume.
- Icteric, hemolytic or lipemic samples, or samples which are turbid or contain fibrin may yield imprecise results. For the use of such samples, please see section Analytical Specificity of this Instructions for Use regarding Endogenous Factors.

Do not use

- Heat-inactivated samples.

■ Precautions

	Follow the user information provided in the User Manual of the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD and in this document.
	Observe the expiration dates specified on the label and do not use after expiration. Reagents from different reagent kits should not be mixed. Do not dissociate the elements of a unit. Avoid any microbic contamination of the reagents. Raw materials of animal and/or human origin contained in the reagents have been tested and/or processed through virus inactivation procedures as far as possible. However, as it is impossible to strictly warrant that such products will not transmit any infectious agents, all raw materials of human or animal origin including the samples to be assayed must be treated as potentially infectious.
 	The generally acknowledged safety precautions and laboratory techniques must be observed when handling reagents and patient samples. • Do not pipet by mouth. • Wash hands after work.
 	Wear protective clothing, protective gloves and safety glasses at work.

	Do not eat, drink or smoke in areas where samples or kit-reagents are handled.
	Remove spills with absorbing paper. All the material used for cleaning up must be disposed of as infectious laboratory waste. Prevent from getting into sewage, water, ground.
	Used reagent plates and reagent kits are disposed of as potential infectious laboratory waste according to local regulations. Empty containers should be returned to local recyclers.

■ Test Principle

The measurement principle of B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD is based on TRACE ™ Technology (Time-Resolved Amplified Cryptate Emission), which measures a fluorescence signal that is emitted from an immunocomplex. The basis of the TRACE ™ Technology is a non-radiative energy transfer from a donor (europium cryptate) to an acceptor (cyanine).

Precise measuring of analyte concentration

The proximity of donor and acceptor when they are part of an immunocomplex and the spectral overlap between donor emission and acceptor absorption spectra, intensify the fluorescence signal of the donor and extend the life span of the acceptor signal, permitting the measurement of temporally delayed fluorescence.

Concretely, when the sample is excited with a nitrogen laser at 337 nm, the donor emits a long-lived fluorescence signal in the millisecond range at 620 nm, while the acceptor generates a short-lived signal in the nanosecond-range measured at 665 nm.

When the two components are bound in an immunocomplex, both the signal amplification and the prolongation of the life span of the acceptor signal occur at 665 nm, so that it can be measured over microseconds.

Reliable prevention of interferences

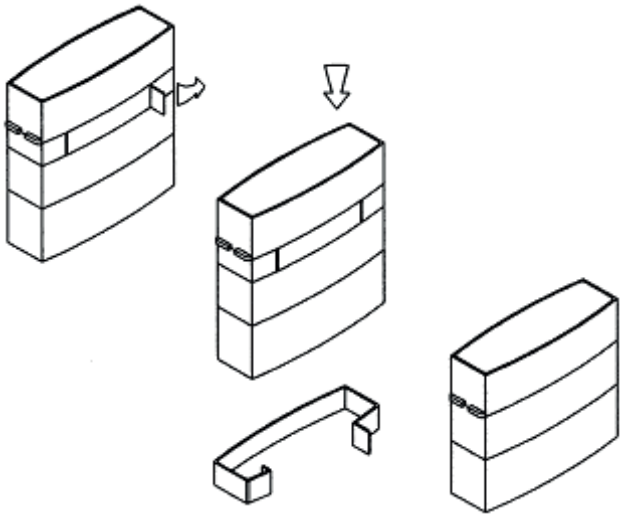
Background fluorescence generated by non-specific signals, e.g. the signals from proteins emitting short-lived fluorescence, interferents or unbound acceptor are eliminated by temporal delay of the fluorescence measurement. The signal generated by the cryptate at 620 nm serves as an internal reference and is measured simultaneously with the long-lived acceptor signal at 665 nm which is the specific signal. Matrix absorption, e.g. from turbid sera, are automatically corrected by means of the internally calculated ratio of the intensities at these wavelengths.

■ Assay Procedure

Reagent Unit

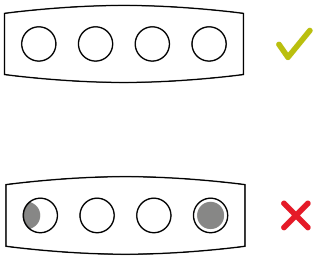
After unpacking the reagent unit, proceed as follows:

- Remove the security band from the reagent pack.
- Push in the lid by pressing it firmly (see diagram below).



Opening the Kit

- Check that the aluminum foil is correctly removed and has not fallen into the vial after opening the reagent unit. The aluminum foil should be placed on the sides of each vial and should not be visible from the top of the reagent unit (see diagram below).



Note: Always handle the reagent unit carefully to avoid the generation of foam or bubbles. Such foam/bubbles may interfere with proper detection of reagents but also with the correct dispensing of reagents. To avoid the presence of foam/bubbles, allow the kit to stand 10 min within B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD before use.

- Register the reagent unit via bar code with the instrument. The instrument stores the required information.
- Each reagent unit is individually identified (bar code) and its maximum period of use after opening is controlled by the instrument.
- Place the reagent unit in the space provided (after it has been opened, the reagent unit may be stored on the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD).
- The instrument identifies whether the reagent unit contains ready to use or freeze dried components.
- Start the reconstitution as requested by the instrument. After the reagent kit reconstitution, allow the kit to stand for 10 min inside B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD, and check carefully that there is no foam or bubbles before use.
- Calibrators, controls and/or patient samples can be placed on the sample carousel for further processing.

The following steps are carried out by the instrument:

- Conjugates and sample are dispensed into the reaction plate and the signal emitted is measured periodically.

Calibration CAL

- A calibration must be carried out for every new reagent lot, then repeated regularly. B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD automatically indicates when calibration is required. Both the stored and recalibrated standard curve may be displayed on the screen.
- Reconstitute each vial with the volume of distilled water indicated on the vial label.

- Allow 15 min for complete dissolution of the lyophilisate.
- Mix gently after reconstitution avoiding foaming. The use of a roller mixer is not recommended.
- Controls must be run after each calibration.
- Use the calibrator/calibrators only once.
- In-use stability: do not leave the calibrator/calibrators at room temperature or on the carousel for more than 5 hours.
- The calibrator bar code card must be read in for each new lot of calibrator.
- For further information, see the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual.

Control

CONTROL

- Controls must be run after each calibration and for each newly installed reagent kit. It is recommended that controls are run at least once a day.
- A control tube is processed like a sample tube.
- Reconstitute each vial with the volume of distilled water as stated on the vial label.
- Allow 15 min for the complete dissolution of the lyophilisate.
- Mix gently after reconstitution avoiding foaming. The use of a roller mixer is not recommended.
- In-use stability: after reconstitution, do not keep a vial more than 2 hours at 18-25 °C or 24 hours at 2-8 °C.
- It is recommended that the contents of a reconstituted vial be divided into aliquots which may then be stored frozen at -20 °C or lower for a maximum period of 1 month.
- Use one of the aliquots immediately for measurement.
- After thawing an aliquot at room temperature, mix gently avoiding foaming and use immediately for measurement. The use of a roller mixer is not recommended.
- Once thawed, a control aliquot must not be refrozen.
- The bar code stick-on labels are used for identifying the controls when assayed on B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD.
- The control kit bar code card must be scanned for each new lot of control.
- For further information see B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD user manual.

■ Result Calculation

A master curve is established with each reagent lot. The parameters defining this master curve are imported into the B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD software when the reagent lot is registered by scanning the barcode card provided with the reagent kit. For every reagent lot, the master curve must be re-adjusted by calibration and repeated regularly.

B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD automatically indicates when calibration is required. Both, the stored and recalibrated master curve can be displayed on the screen. A normalized fluorescence ratio 665/620 nm is calculated at the end of the incubation. The sample concentration is then automatically calculated by the analyzer using the stored recalibrated master curve.

■ Quality Control Procedures

Good laboratory practice requires that control samples are measured regularly to ensure the accuracy and precision of the assay. It is recommended to run controls at least once a day and after each calibration. These samples must be processed exactly the same way as the patient samples and it is recommended that the results be analyzed using appropriate statistical methods.

If desired, B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD can monitor the day-to-day performance of the assay using a pre-installed statistical package with Levy-Jennings graph function.

It is necessary to comply with national quality assurance guidelines for quantitative tests in the medical laboratory (current version). For instance, test accuracy and precision should be monitored utilizing laboratory in-house and/or commercially available control materials. The laboratory must establish and follow their corrective measures to be taken if the control results fall outside the defined limits.

■ Main Assay Features

Sample volume	14 µL
Incubation time	29 min
Results are given in	ng/mL
Conversion factor	n/a
Linear direct measuring range	16.6...3 000ng/mL
Upper measuring range with automatic dilution	up to 1 000 000 ng/mL
Sample type	serum, EDTA plasma
Kit stability on board (in-use stability)	29 days
Calibrator	1 point
Calibration stability	15 days
Assay principle	sandwich

If the samples are taken on EDTA plasma, the values measured with B·R·A·H·M·S CgA II KRYPTOR are systematically lower compared to serum: Spearman correlation coefficient: r = 0.99, Passing Bablok regression EDTA plasma vs. serum: y = 0.85 + 0.66x. Other method may exhibit different impact of matrix on CgA results (e.g. higher values in EDTA plasma compared to serum.)

The concentration of Chromogranin A in a given patient sample, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.

■ Clinical Performance Characteristics

Reference Range

This study was performed on the B·R·A·H·M·S KRYPTOR compact PLUS. Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

A reference range was established based on serum samples from 176 self-declared healthy individuals.

The 95th percentile has been found at 101.9 ng/mL (median: 45.8 ng/mL).

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

Diagnostic Sensitivity and Specificity

CgA II in GEP-NETs

A prospective, multi-center, observational study with 153 evaluable neuroendocrine tumor patients was performed to validate the performance of B·R·A·H·M·S CgA II KRYPTOR assay in monitoring grade 1 and grade 2 GEP-NET progressive or non-progressive disease within 32 months. Course of disease was assessed by standard imaging (CT/MRI scans) and tumors were classified by RECIST 1.1 criteria for progression (progressive disease) vs. no progression (complete response, partial response or stable disease).Change of CgA was calculated from measurements at consecutive routine monitoring visits within a typical interval of 3-6 months and was considered test-positive if serum CgA concentration increased by more than 50% to an absolute value greater than 100 ng/mL. A positive CgA-change test was shown to be significantly associated with tumor progression ($p < 0.001$) and the following diagnostic performance measures for tumor progression were obtained:Clinical specificity = 93.4%; clinical sensitivity = 34.4%; PPV = 57.9%; NPV = 84.3%

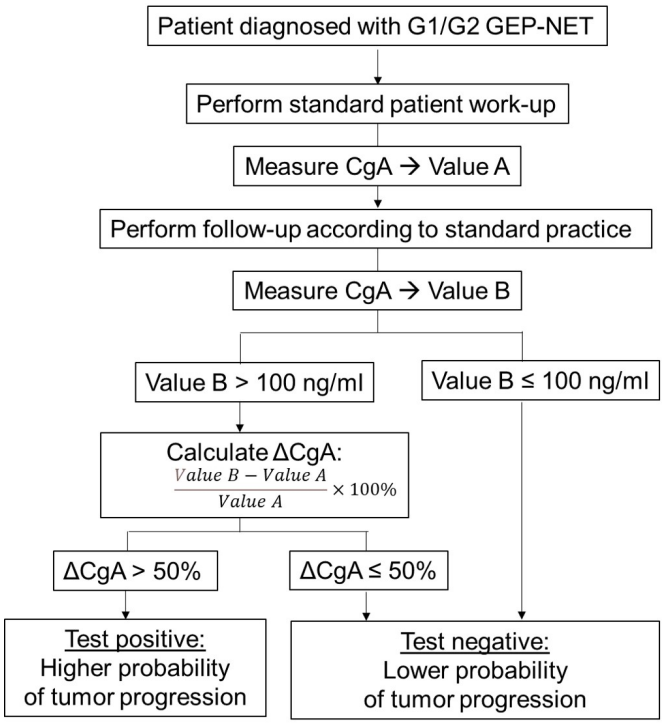
The change of CgA concentration over time provides diagnostic information whether a tumor progression has occurred.

- ΔCgA > 50% and CgA > 100 ng/ml:

An increase of CgA serum concentrations of more than 50% to a value of greater than 100 ng/ml between consecutive monitoring visits defines a positive test result representing a higher probability that a tumor progression has occurred

- ΔCgA ≤ 50% or CgA ≤ 100 ng/ml:

A change of CgA serum concentrations of equal or less than 50% increase between monitoring visits or to a value of 100 ng/ml or less defines a negative test result representing a lower probability that a tumor progression has occurred



CgA II in Pheochromocytomas

44 patients with histologically proved adrenal pheochromocytoma, 100 healthy subjects and 148 patients affected by essential hypertension were included in a clinical study with the aim to assess diagnostic performance of CgA for aid in diagnosis of pheochromocytoma. CgA measured in serum samples showed 95% sensitivity and 96% specificity for detection of pheochromocytoma. By combining CgA with measurement of metanephrines, 100% sensitivity was demonstrated for detection of pheochromocytoma. Serum CgA levels were measured by an established assay demonstrating substantial equivalence to B·R·A·H·M·S CgA II KRYPTOR [14].

Comparable results were observed in a study where plasma samples were measured with B·R·A·H·M·S Chromogranin A KRYPTOR in patients with pheochromocytoma (N = 20) and patients without tumors (N = 32): Sensitivity was 100% for detection of pheochromocytoma at the cut-off 85 µg/L CgA [15].

Interpretation of Results

CgA values need to be evaluated in combination with clinical symptoms and/or other laboratory parameters. Values higher than the 95th percentile, as stated above, should be considered pathological under consideration of the limitations of the assay. Thresholds for medical decisions can vary depending on the intended use. CgA value changes over time can give information on pathological state when used for follow-up and monitoring. CgA results should be interpreted with respect to the intended use.

Method Comparison

A comparison of the B·R·A·H·M·S CgA II KRYPTOR assay (y) with a commercially available Chromogranin A assay (x) using clinical serum samples gave the following correlation [ng/mL]:

Number of samples measured: 98

Comparative Assay: B·R·A·H·M·S Chromogranin A KRYPTOR

Passing/Bablok

y= 1.03x + 6.93

r (Spearman) = 0.99

The sample concentrations were between 17.9 and 2 197 ng/mL.

A comparison of the B·R·A·H·M·S CgA II KRYPTOR assay (y) with a commercially available Chromogranin A assay (x) using clinical serum samples gave the following correlation [ng/mL]:

Number of samples measured: 139

Comparative Assay: Cisbio CGA-ELISA

Passing/Bablok

y= 0.87x -7.53

r (Spearman) = 0.96

The sample concentrations were between 23.4 and 3 728 ng/mL.

■ Analytical Performance Characteristics

Representative performance data are provided in this section. The provided data represents the highest results obtained across all types of instruments. Assay results obtained in individual laboratories may vary from data presented.

Accuracy

Trueness / Bias

The maximum bias allowed to the internal standards concentration value (trueness) of this device is ≤ ± 15%.

Reference material	internal standard material
Concentration range	55 - 3 500 ng/mL
Number of dilutions	6
Passing/Bablok regression	y = 1.009x – 1.567
Slope	1.01
Slope specification	1.00 ± 15%
Observed bias	0.9%

Precision / Repeatability

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures).

Samples have been measured on up to 20 days, with 2 runs per day in 2 replicates using 3 reagent lots.

Results from this study are summarized in the following tables:

Sample	Mean [ng/mL]	Repeatability %CV
Sample 1	34.6	3.7
Sample 2	91.5	2.2
Sample 3	482	1.7
Sample 4	1 825	1.3
Sample 5	25 491	1.9

Sample	Mean [ng/mL]	Within-lab precision %CV
Sample 6	51.9	12.6
Sample 7	91.5	8.8
Sample 8	132	7.1
Sample 9	1 577	7.7
Sample 10	23 491	8.7

Precision / Reproducibility

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP05-A3 (Evaluation of Precision of Quantitative Measurement Procedures).

Samples have been measured on 5 days, with one run per day, in 5 replicates on 3 instruments.

Results from this study for selected samples are summarized in the following table:

Sample	Mean [ng/mL]	Reproducibility %CV
Sample 11	34.0	6.8
Sample 12	87.3	4.0
Sample 13	151	4.1
Sample 14	2 078	2.9
Sample 15	20371	3.6

Analytical Specificity

Potentially interfering substances (i.e. medicinal drugs, nutritional supplements) and/or cross-reacting factors are determined with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP07-A03 (Interference Testing in Clinical Chemistry) .

The following substances were tested for their potential to interfere with assay performance. They were added to 3 (for endogenous factors) or 2 (other interferences) serum samples with Chromogranin A concentrations of 80, 120 and 1 100 ng/mL (for endogenous factors) and 94 and 619 ng/mL (for other interferences) and were found to not interfere or cross-react at the concentrations indicated below:

Endogenous factors

Analyte	Max. Concentration tested
Albumin	50 g/L
Bilirubin	500 mg/L
HAMAs	300 µg/L
Hemoglobin	5 g/L
Rheumatoid factors	1 000 kIU/L
Triglycerides	5 g/L

Medicinal drugs and nutritional supplements

Drug/ substance	Max. Concentration tested
Acetaminophen	238.3 mg/L
Alprazolam	6.0 mg/L

Drug/ substance	Max. Concentration tested
Amlodipine	100.2 µg/L
Arbiraterone	1.22 mg/L
Aspirin	546.6 mg/L
Bevacizumab	720 mg/L
Bicalutamide	339 mg/L
Biotin	3 510 ng/mL
Buserelin	25 ng/mL
Capecitabine	2.85 g/L
Carboplatin	1 g/L
Cisplatin	2 g/L
Degarelix	0.21 mg/L
Dexamethason	1.2 mg/dL
Enzalutamid	90.9 mg/L
Estramustine phosphate	200 µg/mL
Etoposide	114 mg/L
Everolimus	6 mg/L
Fish Oil	2.4 g/L
Fluorouracil	684 mg/L
Hydrochlorothiazide	6.0 mg/L
Hydrocodone	200.3 µg/L
Hydroxyflutamide	10 µg/mL
Ibuprofen	499.6 mg/L
Interferon (IFN-α-2b)	3 000 kU/L
Lanreotide	72 mg/L
Leuprorelin acetate	100 µg/mL
Lisinopril	300.4 µg/L
Lorazepam	998.3 µg/L
Methotrexate	1 360 mg/L
Metoprolol	5.0 mg/L
Multivitamins: Vitamin A Vitamin C Vitamin D Vitamin E Thiamin (B1) Riboflavin (B2) Niacin Vitamin B6 Vitamin B12	 16.7 kIU/L 1 000 mg/L 5.33 kIU/L 100.0 IU/L 200 mg/L 250 mg/L 170 mg/L 170 mg/L 3 333 µg/L
Octreotide	12 mg/L
Oxaliplatin	96.9 mg/L
Oxycodone	500.9 µg/L
Pancrelipase	480 KU/L
Prednisolone	1.2 mg/L
Sunitinib	22.5 mg/L
Temozolomide	228 mg/L
Temsirolimus	15 mg/L

Cross-reactants

Cross-reactants	Concentration tested [nmol/L]	Observed cross-reactivity
Parastatin (porcine); amino acid 347 to 365	100	0.08%
Catestatin (human); amino acid 352 to 372	452	0.15%
Pancreastatin (human); amino acid 250 to 301	182	2.1%
Vasostatin I (human); amino acid 17 to 76	9	6.7%
Vasostatin II Cterm (human); amino acid 97 to 131	15	1.8%
Vasostatin II (human); amino acid 19 to 131	5	21.6%
Chromostatin (bovine); amino acid 124 to 143	10	11.0%
Chromogranin A protein fragment (human); amino acid 260 to 454	217	0.19%
Chromogranin B (Secretogranin 1) (human); amino acid 21 to 677	72	1.3%
Chromogranin C (Secretogranin 2) (human); amino acid 1 to 617	148	0.72%
WE14 (human); amino acid 315 to 329	606	0.18%

Metrological Traceability

An International CgA reference preparation was not available. Therefore, the assay was calibrated using a freeze-dried reference preparations of synthetic CgA as standards. This calibration is verified on a regular basis by regression analysis and comparison to theoretically expected results using up to 4 lots. Quality Control of new calibrator manufacturing ensures reproducibility and stability of calibration along time (according to internal procedure).

Measuring Range

Linearity

A study was performed with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP06-A guideline (Evaluation of the Linearity of Quantitative Measurement Procedures).

The method has been demonstrated to be linear from LoQ to 1 000 000 ng/mL, with a maximum bias of +/- 20% in this interval.

Patient samples can be diluted if required.

Limit of Detection

The **Limit of Detection (LoD)** is calculated with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures).

The LoD has been assessed as being 14.6 ng/mL.

Limit of Quantitation

The **Limit of Quantitation (LoQ)** is calculated with guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures), and a Total Error (imprecision and bias) goal of 40% calculated using the Westgard Model.

The LoQ has been assessed as being 16.6 ng/mL.

Measuring Range

The measuring range of the B·R·A·H·M·S CgA II KRYPTOR assay is from LoD up to 3 000 ng/mL without dilution.

Measuring range with automatic dilution is up to 1 000 000 ng/mL.

High Dose Hook Effect

No “High Dose Hook” effect can be observed up to 1 000 000 ng/mL.

■ Important Notice

Any serious incident that has occurred in relation to the device shall be reported to B·R·A·H·M·S GmbH 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 on EUDAMED and upon request.

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■ Symbols

Symbols used in Instructions for Use and Product Labelling of B·R·A·H·M·S KRYPTOR compact PLUS/ KRYPTOR GOLD products.

Symbol	Usage	Symbol	Usage	Symbol	Usage
Intended Use	Reference to the Intended use of the Medical Device	IVD	In Vitro Diagnostic Medical Device	LOT	Batch Code
CONT	Contents	CAL	Calibrator	CONTROL	Control
BUF	Buffer	SOLN 1	B·R·A·H·M·S KRYPTOR compact Solution 1/B·R·A·H·M·S Solution 1 KRYPTOR GOLD	SOLN 2	B·R·A·H·M·S KRYPTOR compact Solution 2/B·R·A·H·M·S Solution 2 KRYPTOR GOLD
SOLN 3	B·R·A·H·M·S KRYPTOR compact Solution 3/B·R·A·H·M·S Solution 3 KRYPTOR GOLD	SOLN 4	B·R·A·H·M·S KRYPTOR compact Solution 4/B·R·A·H·M·S Solution 4 KRYPTOR GOLD	CONT BAGS	Bags contained
BAGS	Bags	CONT PLATES	Plates contained	PLATES	Plates
CONT VIALS	Vials contained	VIALS	Vials	VIAL	Vial
H2O	Use given volume of distilled water (conductivity of less than 50 µS/cm is recommended) for reconstitution, e.g. 0.75 mL	LYOPH	Lyophilized, Freeze Dried	RCNS	Reconstitute
	Manufacturer		Used by		Green Dot according to German Law
®	Registered Trade Mark	™	Trade Mark	REF	Article Number/Catalogue Number
 50	Contains sufficient for (Number of) tests, e.g. 50		Consult Instructions for Use		Waste
	Caution		Wear Protective Gloves		Wear Safety Glasses
	Wash hands		General Regulatory Sign		General Prohibitive Sign
	Do not Smoke		Do not Eat and Drink		GHS05, Corrosive
	GHS07, Harmful		GHS03, Oxidizing		Accidental Release Measures
TRACE	Trade Mark for TRACE™-technology	CE	CE Conformity Marking According to Regulation (EU) 2017/746 on In Vitro Diagnostics Medical Devices (Class A)	CE 0197	CE Conformity Marking According to Regulation (EU) 2017/746 on In Vitro Diagnostics Medical Devices, with Reg. Number of Notified Body (Class B, C, D)
	Temperature Limitation		Do not Reuse		