



CGRP (human)

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CGRP (human) ELISA kit #A05481.96 wells

For research laboratory use only Not for human diagnostic use

This assay was developed & validated by Bertin Bioreagent

Fabriqué en France Made in France



#A11481

Version: 0118

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96 wells Storage: -20°C

Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
CGRP precoated 96-well Strip Plate	Blister with zip	A08481.1 ea	1	-
CGRP (human) Tracer	Green	A04481.100 dtn	1	Lyophilised
CGRP (human) Standard	Blue with red septum	A06481.1 ea	2	Lyophilised
CGRP (human) Quality Control	transparent	A10481.1ea	2	Liquid
EIA Buffer	Green with red septum	A07000.1 ea	1	Lyophilised
Wash Buffer	Blue	A17000.1 ea	1	Lyophilised
Tween 20	Silver	A12000.1 ea	1	Liquid
Ellman's reagent 49+1	Black with red septum	A09000_49+1.10 0 dtn	2	Lyophilised
Technical Booklet	-	A11481.1 ea	1	-
Well cover Sheet	-	-	1	-

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area where kit reagents are handled
- Avoid splashing

The total amount of reagents contain less than 100 μ g of sodium azide. Flush the drains thoroughly to prevent the production of explosive metal azides.

Wearing lab gloves, laboratory coat and eye protection glasses is recommended when assaying kit materials and samples.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), which is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

Acetylcholinesterase AChE Technology

Acetylcholinesterase (AChE), the enzymatic label for EIA, has the fastest turnover rate of any enzymatic label. This specific AChE is extracted from the electric organ of the electric eel, *Electrophorus electricus*, and it is capable of providing a rapid catalytic turnover during the generation of the electrochemical discharges. The use of AChE as enzymatic label for EIA is patented by the French academic research Institute CEA *[1, 2, 3]*, and Bertin Bioreagent has expertise to develop and produce EIA/ELISA kits using this technology.

AChE assays are revealed with Ellman's reagent, which contains acetylthiocholine as a substrate. The final product of the enzymatic reaction (5-thio-2-nitrobenzoic acid), is bright yellow in color and can be read at 405-414 nm using a spectrophotometer. AChE offers several advantages over other commonly used enzymes used in EIAs:

- Kinetic superiority and high sensitivity: AChE shows true first-order kinetics with a turnover of 64,000 sec⁻¹. That is nearly 3 times faster than Horse Radish Peroxidase (HRP) or alkaline phosphatase. AChE provides greater sensitivity than other labeling enzymes.
- Low background: Non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent.
 Thus, AChE ensures a very low background and an increased signal/noise ratio compared to other

- substrate of enzymes that are inherently unstable.
- Wide dynamic range: AChE is a stable enzyme and its activity remains constant for many hours.
 Unlike other enzymes, AChE has substrate that is not suicidal which permits simultaneous assays of high and low concentration samples.
- Versatility: AChE is a completely stable enzyme, unlike peroxidase which is suicidal. The accidentally dropped plate containing AChE substrate (Ellman's reagent) does not need to be discarded and experiment can be continued by adding washing buffer and fresh Ellman's reagent into the plate wells. As an option Otherwise, plate can be stored at +4°C containing washing buffer while waiting for technical advice from the Bioreagent Department.

CGRP

Calcitonin Gene Related Peptide (CGRP) is a potent vasodilatator and also elicits a nuber of other biological effects.

Average plasma levels of CGRP have been reported to be from 0.8 pmol/L to 71 pmol/L (3pg/mL to 269 pg/mL) in normal subjects. Increases in circulating CGRP levels have been noticed during hemodialysis, pregnancy, exacerbation of asthma and in cases of medulalary thyroid carcinoma.

Principle of the assay

The enzymatic immunoassay (EIA/ELISA) is based on a sandwich technique. Wells of supplied plate are coated with a monoclonal antibody specific to CGRP.

CGRP introduced into the wells (standard or sample) is bound by the monoclonal antibody coated on the plate and is then detected by an acetylcholinesterase (AChE) – Fab' conjugate also specific of CGRP.

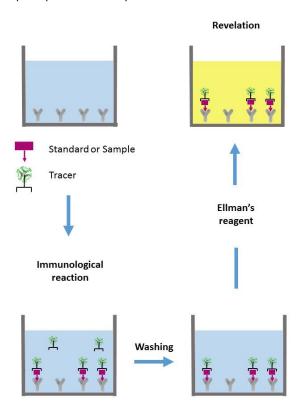
The two antibodies then form a sandwich by binding on different epitopes of the CGRP.

The sandwich is immobilised on the plate where excess reagents are washed away.

The concentration of CGRP is determined by measuring the enzymatic activity of immobilized tracer using Ellman's reagent. AChE tracer acts on Ellman's Reagent to form a yellow compound that strongly absorbs at 405 nm or at 414 nm.

The intensity of colour, which is determined by spectrophotometry, is proportional to the amount of CGRP present in the well during the immunological reaction

The principle of the assay is summarised below:



Assay characteristics

Validated for use:

- in buffer
- in human plasma (without extraction, using a standard curve in plasma).

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography [7,8]

- Assay validation data: ask Bertin Bioreagent (tech@bertin-bioreagent.com) or your local distributor for a copy of the validation data in EIA buffer.
- Limit of detection (LOD): 0.7 pg/mL (in EIA buffer) and 2 pg/mL (in plasma), calculated as the concentration of CGRP corresponding to the NSB average (n=8) plus three standard deviations.

Cross-reactivity

CGRP- α/β (human)	100 %	CGRP (8-37)	<0.01 %
CGRP-I/II (human)	100 %	Amylin	<0.01 %
CGRP-α/β (rat)	120%	Calcitonin	<0.01 %
CGRP-I/II (rat)	120%	Substance P	<0.01 %

Materials and equipment required

In addition to standard laboratory equipment, the following materials are required:

For the sample preparation:

- C-18 reverse phase cartridges or Oasis[®] HLB Extraction cartridges
- Methanol
- Acetic acid

For the assay:

- Precision micropipettes (20 to 1000 μL)
- Spectrophotometer plate reader (405 nm or 414 nm filter)
- Microplate washer (or wash bottles)
- Orbital microplate shaker

- Multichannel pipette and disposable tips 30-300μL
- UltraPure water #A07001.1L
- Polypropylene tubes



Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).

Do not use distilled water, HPLC-grade water or sterile water.

 UltraPure water may be purchased from Bertin Bioreagent (item #A07001.1L).

Sample preparation

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C or at -80°C prior the use with the assay.

Nervous tissues such as cerebrospinal fluid may be assayed directly if diluted more than 1:20 in EIA buffer. Other nervous tissues such as spinal cord may be assayed after extraction procedure. Basically, the procedure is to homogenize the tissue in 2 N acetic acid, heat at 90°C for ten minutes, centrifuge, dry the supernatant, and then dissolve in EIA buffer.

Plasma and serum samples should be measured after extraction (see extraction protocol below), or without any

extraction procedure.

In this second case, CGRP standard and Quality Control need to be reconstituted with plasma or serum that is free from CGRP (human) instead of the EIA buffer as mentioned in reagent preparation section (CGRP standard and Quality Control). If you don't have plasma or serum that is free of CGRP (human), Bertin Bioreagent offers CGRP affinity sorbent containing anti-CGRP monoclonal antibody (the same as the one coated on the wells) under cat# A19482.

Whole blood, as well as other heterogeneous mixtures such as lavage fluids and aspirates should be purified (see extraction protocol below) before addition to the assay wells.

Extraction protocol

- Activate a 1 mL C-18 reverse phase cartridge or an Oasis® HLB Extraction cartridge (Bertin Bioreagent cat# D30005 or Waters cat# WAT094226-HLB-3cc) by first passing 5 mL methanol and then 10 mL UltraPure water through the cartridge. The reverse phase cartridge (RPC) may be stored with the water present.
- Dilute the sample at 1:4 with 4% acetic acid.
- Pass 1 ml of sample slowly (about 2 mL/minute) through the cartridge.
- Wash the cartridge with 10 mL of 4% acetic acid.
- Prepare 3 mL of methanol: 4% acetic acid aqueous solution (90:10, v/v). Elute the CGRP by

passing the methanol:water solution through the cartridge 1 ml at a time. Be certain to pause between each ml of solution as the reproducibility of the recovery is increased by the care taken during this step.

- Dry the sample by vacuum centrifugation.
 Reconstitute the sample with a volume of EIA buffer equal to the original sample volume.
- Assay the aliquots of the sample and use the results to calculate the recovery.

Recovery and calculation

To determine the recovery, the sample may be split into two equal aliquots and one spiked with a known amount of CGRP (approximately equal to the expected amount in the sample).

The recovery will be determined after purification by comparing the concentration of the spiked and unspiked samples. Either the original concentration of the sample or the recovery factor can be determined by solving the following equations simultaneously:

z = recovery factor

X/a =original concentration of the unspiked sample in a volume known (a)

(X+Y)/b = concentration of the spiked sample (pg/mL) after adding a known amount (Y) in a final volume (b)

The concentration of the unspiked and spiked samples

determined by the EIA are respectively equal to (X/a)z and [(X+Y)/b]z.

Example

- Volume of the unspiked sample: a = 1 mL
- Final volume of the spiked sample: b = 2 mL
- Concentration determined by EIA for the unspiked sample: (X/a)z = 8 pg/mL
- Concentration determined by EIA for the spiked sample: [(X+Y)/b]z = 16 pg/mL
- Quantity of spike: Y = 30 pg in 1mL

```
Xz = 8 hence z=8/X [(X+30)/2]z = 16 hence [(X+30)]z = 32 thus, [(X+30)]8/X = 32 X+30 = 4X 3X = 30 X = 10 and Xz = 8, so z = 0.8
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Note

To minimise the calculations, the standard should be concentrated enough so that the addition of the standard does not alter the volume of the sample (a = b) to any great degree (i.e., assumption is made that the volume is not changed by the addition of the standard).

Reagent preparation

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 36 samples in duplicate according to suggested plate layout.

An additional vial of Standard and Quality Control are provided in case you need to perform 2 assays with the kit.

All reagents must be brought to room temperature (around +20°C) prior the use in assay.

EIA Buffer

Reconstitute the EIA Buffer #A07000 with 50 mL of UltraPure water. Allow buffer to stand for 5 minutes or until it is completely dissolved. Mix buffer thoroughly by gentle inversions.

Stability at 4°C: 1 month.

CGRP (human) Standard

Reconstitute the CGRP (human) Standard vial #A06481 with 1 mL of EIA buffer. Allow standard to stand for 5 minutes or until it is completely dissolved. Mix standard thoroughly by gentle inversions.

The concentration of the first standard (S1) is 1000 pg/mL. Prepare seven polypropylene tubes (for the other standards) and add 500 μ L of EIA Buffer into each tube. Then prepare the standards by serial dilutions as indicated in following

table. Mix each tube thoroughly before the next transfer.

Standard	Volume of Standard	Volume of EIA Buffer	Standard concentration
S1	-	-	1000 pg/mL
S2	500 μL of S1	500 μL	500 pg/mL
S3	500 μL of S2	500 μL	250 pg/mL
S4	500 μL of S3	500 μL	125 pg/mL
S5	500 μL of S4	500 μL	62.5 pg/mL
S6	500 μL of S5	500 μL	31.25 pg/mL
S7	500 μL of S6	500 μL	15.53 pg/mL
S8	500 μL of S7	500 μL	7.81 pg/mL

Stability at 4°C: 24 hours

CGRP (human) Quality Control

Reconstitute one vial with 1 mL of EIA buffer. Allow quality control to stand for 5 minutes or until it is completely dissolved. Mix quality control thoroughly by gentle inversions.

Stability at 4°C: 24 hours

CGRP (human) Tracer

Reconstitute one vial with 10 mL of EIA Buffer. Allow tracer to stand for 5 minutes or until it is completely dissolved. Mix tracer thoroughly by gentle inversions.

Stability at +4°C: 1 month

Wash Buffer

Dilute 1 mL of concentrated Wash Buffer #A17000 with 400

mL of UltraPure water. Add 200 μ L of Tween 20 #A12000. Use a magnetic stirring bar to mix the content. Note that concentrated wash buffer is also used for Ellman's reagent preparation.

Stability at +4°C: 1 week.

▶ Ellman's Reagent

5 minutes before use (development of the plate), reconstitute one vial of Elman's Reagent #A09000_49+1 with 49 mL of UltraPure water and 1 mL of **concentrated** Wash Buffer#A17000. The tube content should be thoroughly mixed.

Stability a +4°C and in the dark: 24 hours

Assay procedure

It is recommended to measure the samples in duplicate following the instruction below.

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.

Open the plate pouch and select enough strips for your assay. Place unused strips back in the pouch.

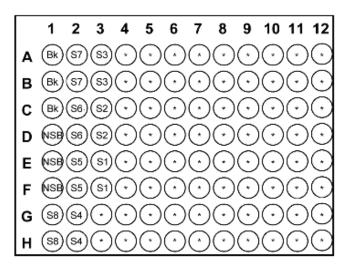
Stability at +4°C: 1 month.

Rinse each well 5 times with Wash Buffer (300 µL/well).

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and blot the last drops by tapping it on paper towels.

Plate set-up

A plate set-up is suggested hereafter. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.



Bk: Blank S1-S8: Standards 1-8

NSB: Non Specific Binding *: Samples or Quality Controls

Pipetting the reagents

Samples and reagents must reach room temperature prior performing the assay.

Use new tips to pipet buffers, standards, samples, antibody and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

> EIA Buffer

Dispense 100 µL to Non Specific Binding wells (NSB) wells.

CGRP (human) Standard

Dispense $100~\mu L$ of each of the eight standards (S8 to S1) in duplicate to appropriate wells.

Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

> CGRP (human) Quality Control and Sample

Dispense 100 μL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA Buffer.

CGRP (human) Tracer

Dispense 100 µL to each well, except Blank (Bk) wells.

Incubating the plate

Cover the plate with cover sheet and incubate for 16-20 hours at +4°C.

Developing and reading the plate

- Reconstitute Ellman's Reagent as mentioned in the reagent preparation section.
- Empty the plate by inverting it. Rinse each well by adding 300 μL of Wash Buffer. Wash strips 3 times, slightly shake them during 2 minutes, then wash 3 times & discard liquid from the wells. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.
- Add 200µL of Ellman's reagent.
- Cover the plate with cover sheet and incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker.
- Wipe the bottom of the plate with a paper towel, and make sure that no liquid has been projected outside the wells.
- Read the plate at a wavelength between 405 and 414nm (yellow colour). After addition of Ellman's Reagent, the absorbance has to be checked periodically (every 30 minutes) until the maximum absorbance has reached a minimum of 0.5 A.U. (blank subtracted).

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)				
	Blank	NSB	Standard	Sample or QC
EIA Buffer	-	100	-	-
Standard	-	-	100	-
Sample or QC	-	-	-	100
Tracer	-	100	100	100
Cover plate, incubate 16-20 hours at +4°C				
Wash strips 3 times, slightly shake them during 2 min, then wash 3 times & Discard liquid from the wells & dry on absorbent paper				
Ellman's reagent 200				
Cover plate, incubate at room temperature in the dark using an orbital microplate				
Read the plate at 405 nm or at 414 nm				

Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate.

- Calculate the average absorbance for each NSB, standards, QC and samples.
- For each standard, plot the absorbance (y axis) versus the concentration (x axis) graph. Draw a best-fit line through the points.
- To determine the concentration of samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of unknown samples.
- Samples with a concentration greater than 1000 pg/mL must be re-assayed after dilution in EIA Buffer.
 - Most plate readers come with a curve-fitting software pre-installed that is capable of generating

graphs (logit/log or 4-parameter logistic fit 4PL). It is highly recommended to use this software if available on the device. Refer to it for further information.



2 vials of Quality Control are provided with this kit. Your standard curve is validated only if the calculated concentration of the Quality Control obtained with the assay is +/- 25% of the expected concentration (see the label of the QC vial)

Acceptable range

- NSB absorbance < 0.06 A.U.
- Limit of detection < 10 pg/mL
- QC ±25% of the expected concentration (see the label of the QC vial)

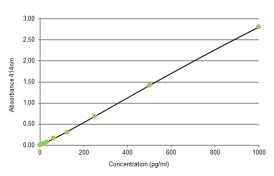
Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.

These data were obtained using all reagents as supplied in this kit under the following conditions: 60 minutes developing at room temperature, reading at 414 nm. A spline line fitting was used to determine the concentrations.

Standard	CGRP (pg/mL)	Absorbance A.U.
S1	1000	2.808
S2	500	1.42
S3	250	0.686
S4	125	0.311
S5	62.5	0.167
S6	31.25	0.0765
S7	15.63	0.0405
S8	7.81	0.018
NSB	0	0.002

Typical CGRP (human) standard curve



Troubleshooting

Absorbance values are too low:

- organic contamination of water,
- one reagent has not been dispensed,
- incorrect preparation/dilution,
- assay performed before reagents reached room temperature,
- · reading time not long enough.

High signal and background in all wells:

- · inefficient washing,
- overdeveloping (incubation time should be reduced),
- high ambient temperature.

High dispersion of duplicates:

- poor pipetting
- irregular plate washing.

If a plate is accidentally dropped after dispatch of the AChE substrate (Ellman's Reagent) or if it needs to be revealed again:

- one only needs to wash the plate, add fresh Ellman's Reagent and proceed with a new development
- otherwise, the plate can be stored at +4°C with wash buffer in wells while waiting for technical advice from the bioreagent department.

These are a few examples of troubleshooting that may occur. If further information or explanation is needed, please contact

Bertin Bioreagent Technical Support by phone on +33 (0)139 306 036, fax +33 (0)139 306 299 or by E-mail tech@bertin-bioreagent.com. Please have batch number of the kit (see outside the box) ready to provide to the technical support.

Bertin Bioreagent offers EIA Training kit #B05005. Feel free to contact our Technical Support. We are always happy to hearing from you.

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Additional readings

List of publications quoting the use of this kit

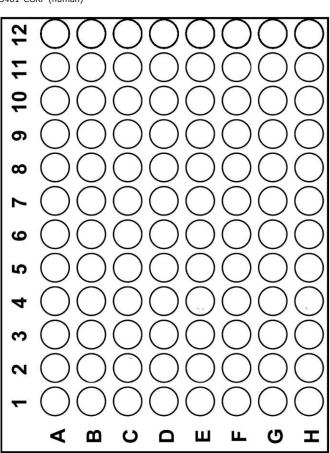
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With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control. We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

Bertin Bioreagent has also a long expertise in developing customized solutions adapted to your need. Feel free to contact us for your special projects!

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Should you need help with an order, you can contact our customer service by emailing to order@bertin-bioreagent.com

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