CGRP (human)
Bertin Pharma also markets pre-analytical products, EIA kits, antibodies & biochemicals for:

- Cardiology / Hypertension
- Diabetes / Obesity
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- Inflammation
- Pharmacology
- Psychopharmacology
- Nitric Oxide
- Oncology / Apoptosis
- Oxidative injury
- Cell signaling
- Drug metabolism

Do not hesitate to contact our after-sales services for further information at bioreagent@bertinpharma.com
CGRP (human) Enzyme Immunoassay kit
#A05481.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma
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This kit contains:

<table>
<thead>
<tr>
<th>Designation</th>
<th>Colour of cap</th>
<th>Item #</th>
<th>Quantity per kit</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGRP precoated 96-well Strip Plate</td>
<td>Blister with zip</td>
<td>A08481.1 ea</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>CGRP (human) Tracer</td>
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<td>A04481.100 dtn</td>
<td>1</td>
<td>Lyophilised</td>
</tr>
<tr>
<td>CGRP (human) Standard</td>
<td>Blue with red septum</td>
<td>A06481.1 ea</td>
<td>2</td>
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<tr>
<td>EIA Buffer</td>
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<td>A07000.1 ea</td>
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<tr>
<td>Wash Buffer</td>
<td>Blue</td>
<td>A17000.1 ea</td>
<td>1</td>
<td>Lyophilised</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Silver</td>
<td>A12000.1 ea</td>
<td>1</td>
<td>Liquid</td>
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<tr>
<td>CGRP (human) Quality Control</td>
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<td>Liquid</td>
</tr>
<tr>
<td>Ellman’s Reagent_49+1</td>
<td>Black with red septum</td>
<td>A09000_49+1.100 dtn</td>
<td>2</td>
<td>Lyophilised</td>
</tr>
<tr>
<td>Technical Booklet</td>
<td>-</td>
<td>A11481.1ea</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Well cover sheet</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Quality Control and one of Ellman’s Reagent.
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 in which kit reagents are handled
- Avoid splashing

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

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

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that 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 is capable of massive catalytic turnover during the generation of the electrochemical discharges. The use of AChE as enzymatic label for EIA has been patented by the French academic research Institute CEA [1, 2, 3], and Bertin Pharma, formerly known as SPI-Bio, has expertise to develop and produce EIA 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 and can be read at 405-414 nm. AChE® offers several advantages compared to enzymes conventionally 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 Horseradish Peroxidase (HRP) or alkaline phosphatase. AChE® allows a greater sensitivity than other labeling enzymes.

> **Low background**: non-enzymatic hydrolysis of acetylthiocholine in buffer is essentially absent. So, AChE® allows a very low background and an increased signal/noise ratio compared to other substrate of enzymes which is inherently unstable.
> **Wide dynamic range:** AChE® is a stable enzyme and its activity remains constant for many hours as, unlike other enzymes, its substrate is not suicidal. This permits simultaneous assays of high diluted and very concentrated samples.

> **Versatility:** AChE® is a completely stable enzyme, unlike peroxidase which is suicidal. Thus, 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.

### CGRP

Calcitonin Gene Related Peptide (CGRP) is a potent vasodilator, and also elicits a number of other biological effects.

Average plasma levels of CGRP have been reported to be from 0.8 pmol/L to 71 pmol/L (3 pg/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 medullary thyroid carcinoma.
Principle of the assay

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the microtiter plate supplied with the kit are coated with a monoclonal antibody specific of CGRP.

This antibody will bind any CGRP introduced in the wells (sample or standard). An acetylcholinesterase (AChE) - Fab’ conjugate, which binds selectively to a different epitope on the CGRP molecule, is also added to the wells.

This allows the two antibodies to form a sandwich by binding on different parts of the human CGRP molecule.

The sandwich is immobilised on the plate so the excess reagents may be washed away.

The concentration of human CGRP is then determined by measuring the enzymatic activity of the AChE using Ellman’s Reagent. The AChE tracer acts on the Ellman’s Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the human CGRP present in the well during the immunological incubation.
The principle of the assay is summarised below:

Wells coated with CGRP monoclonal antibody

+ Free CGRP (standard or sample)

AChE Anti-CGRP tracer

Immunological reaction

Reading (414 nm)

AChE AChE AChE

Ellman's reagent

Washing

Incubation
Materials and equipment required

In addition to standard laboratory equipment, the following material is 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 or 414 nm filter)
> Microplate washer (or washbottles)
> Orbitral microplate shaker
> UltraPure water (item number #A07001.1L)
> Polypropylene tubes

Water used to prepare all EIA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces). Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer Acetylcholinesterase (AChE).

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

UltraPure water may be purchased from Bertin Pharma : 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.

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 2N 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 Pharma can provide you with CGRP affinity sorbent containing anti-CGRP monoclonal antibody (the same as the one coated to the well).
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 well.

**Extraction protocol**

- Activate a 1 mL C-18 reverse phase cartridge or an Oasis® HLB Extraction cartridge (Bertin Pharma #D30005 or Waters item #WAT094226-HLB-3cc) by first passing 5 mL of methanol and then 10 mL of UltraPure water through the cartridge. The reverse phase cartridge (RPC) may be stored with the water still present.
- Dilute the sample 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:water with 4% acetic acid (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:

\[ z = \text{recovery factor} \]
\[ \frac{X}{a} = \text{original concentration of the unspiked sample in a volume known (a)} \]
\[ \frac{X+Y}{b} = \text{concentration of the spiked sample (pg/mL) after adding a known amount (Y) in a final volume (b)} \]

The concentration of the unspiked and spiked samples determined by the EIA are respectively equal to \( \frac{X}{a}z \) and \( \frac{(X+Y)}{b}z \).

**Example**

- Volume of the unspiked sample: \( a = 1\text{mL} \)
- Final volume of the spiked sample: \( b = 2 \text{ mL} \)
- Concentration determined by EIA for the unspiked sample: \( \frac{X}{a}z = 8 \text{ pg/mL} \)
- Concentration determined by EIA for the spiked sample: \( \frac{(X+Y)}{b}z = 16 \text{ pg/mL} \)
> Quantity of spike: $Y = 30$ pg in 1mL

$Xz = 8 \Leftrightarrow z = \frac{8}{X}$

$\frac{(X+30)}{2}z = 16 \Leftrightarrow [(X+30)]z = 32$

thus,

$\frac{(X+30)}{8/X} = 32 \Leftrightarrow X+30 = 4X \Leftrightarrow 3X = 30 \Leftrightarrow X = 10$

and

$Xz = 8 \Leftrightarrow z = 0.8$

**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., the assumption is made that the volume is not changed by the addition of the standard).
A05481 - CGRP (human)

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.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Quality Control and one of Ellman’s Reagent.

All reagents need to be brought to room temperature, around +20°C, prior to the assay.

EIA Buffer

Reconstitute the vial #A07000 with 50 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Stability at 4°C: 1 month

CGRP (human) Standard

Reconstitute the Standard vial (item #A06481) with 1 mL of EIA Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. The concentration of the first standard (S1) is 1000 pg/mL.

Prepare seven propylene tubes for the other standards and add 500 µL of EIA Buffer into each tube. Then prepare the standards by serial dilutions as follows:
<table>
<thead>
<tr>
<th>Standard</th>
<th>Volume of Standard</th>
<th>Volume of Assay Buffer</th>
<th>Standard concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-</td>
<td>-</td>
<td>1000 pg/mL</td>
</tr>
<tr>
<td>S2</td>
<td>500 µL of S1</td>
<td>500 µL</td>
<td>500 pg/mL</td>
</tr>
<tr>
<td>S3</td>
<td>500 µL of S2</td>
<td>500 µL</td>
<td>250 pg/mL</td>
</tr>
<tr>
<td>S4</td>
<td>500 µL of S3</td>
<td>500 µL</td>
<td>125 pg/mL</td>
</tr>
<tr>
<td>S5</td>
<td>500 µL of S4</td>
<td>500 µL</td>
<td>62.5 pg/mL</td>
</tr>
<tr>
<td>S6</td>
<td>500 µL of S5</td>
<td>500 µL</td>
<td>31.25 pg/mL</td>
</tr>
<tr>
<td>S7</td>
<td>500 µL of S6</td>
<td>500 µL</td>
<td>15.53 pg/mL</td>
</tr>
<tr>
<td>S8</td>
<td>500 µL of S7</td>
<td>500 µL</td>
<td>7.81 pg/mL</td>
</tr>
</tbody>
</table>

**Stability at 4°C: 24 hours**

**CGRP (human) Quality Control**

Reconstitute one vial with 1 mL of EIA Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

*Stability at +4°C: 24 hours.*

**CGRP (human) Tracer**

Reconstitute one vial with 10 mL of EIA Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

*Stability at +4°C: 1 month.*
**Wash Buffer**

Dilute 1 mL of concentrated Wash Buffer #A17000 with 400 mL of UltraPure water. Add 200 µL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content.

*Stability at +4°C: 1 week*

**Ellman’s Reagent**

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

*Stability at +4°C and in the dark: 24 hours*
Assay procedure

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.

*Stability at +4°C: 1 month.*

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

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

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.
Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet the buffers, standards, samples, tracer, antiserum 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 (NSB) wells.

> **CGRP (human) Standard**
Dispense 100 µL of each of the eight standards S1 to S8 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 samples**
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 the 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 turning it over. Rinse each well five times with 300 µL of Wash Buffer. Wash strips 3 times, slightly shake them 2 min., rewash 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 200 µL of Ellman’s Reagent to each 96 well. Cover the plate with aluminium 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 splashed 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).**
## Enzyme Immunoassay Protocol (volumes are in µL)

<table>
<thead>
<tr>
<th>Volume</th>
<th>Wells</th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
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</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>-</td>
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<td>Standard</td>
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<td>-</td>
<td>100</td>
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<td>-</td>
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<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tracer</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Cover plate, incubate 16-20 hours at +4°C

Wash strips 3 times, slightly shake them 2 min., rewash 3 times & discard liquid from the wells

Ellman’s Reagent | 200

Incubate with an orbital shaker in the dark at RT

Read the plate between 405 and 414 nm
Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of Ellman’s Reagent alone) from the absorbance readings of the rest of the plate. If not, do it now.

- Calculate the average absorbance for each NSB, standard and sample.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your 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 your unknown sample.
- Samples with a concentration greater than 1000 pg/mL should be re-assayed after dilution in EIA Buffer.
- Most plate readers are supplied with curve-fitting software capable of graphing these data (logit/log or 4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Two 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 QC vial)
Acceptable range

- NSB absorbance <60 mAU
- Limit of detection <10 pg/mL.
- QC: +/-25% of the expected concentration (see the label of 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 +20°C, reading at 414 nm. A spline fitting was used to determine the concentrations.

<table>
<thead>
<tr>
<th>CGRP</th>
<th>mAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>2.0</td>
</tr>
<tr>
<td>Standard 1000 pg/mL</td>
<td>2808</td>
</tr>
<tr>
<td>Standard 500 pg/mL</td>
<td>1420</td>
</tr>
<tr>
<td>Standard 250 pg/mL</td>
<td>686</td>
</tr>
<tr>
<td>Standard 125 pg/mL</td>
<td>311</td>
</tr>
<tr>
<td>Standard 62.5 pg/mL</td>
<td>167</td>
</tr>
<tr>
<td>Standard 31.25 pg/mL</td>
<td>76.5</td>
</tr>
<tr>
<td>Standard 15.63 pg/mL</td>
<td>40.5</td>
</tr>
<tr>
<td>Standard 7.81 pg/mL</td>
<td>18.0</td>
</tr>
</tbody>
</table>

**Typical CGRP (human) standard curve**
Assay validation and characteristics

The Enzyme Immunometric assay of CGRP (human) has been validated for its use in buffer and 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].

> The limit of detection (LOD) calculated as the concentration of CGRP corresponding to the NSB average (n = 8) plus three standard deviations, is: 0.7 (in EIA Buffer) and 2 pg/mL (in plasma).

> Quality Control samples intra-assay variations in EIA Buffer (n = 25)

<table>
<thead>
<tr>
<th>CGRP (human)</th>
<th>C.V.</th>
<th>CGRP (human)</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.81 pg/mL</td>
<td>&gt; 25 %</td>
<td>125 pg/mL</td>
<td>3.3 %</td>
</tr>
<tr>
<td>15.6 pg/mL</td>
<td>20.6 %</td>
<td>250 pg/mL</td>
<td>3.0 %</td>
</tr>
<tr>
<td>31.3 pg/mL</td>
<td>7.0 %</td>
<td>500 pg/mL</td>
<td>2.7 %</td>
</tr>
<tr>
<td>62.5 pg/mL</td>
<td>5.2 %</td>
<td>1000 pg/mL</td>
<td>3.4 %</td>
</tr>
</tbody>
</table>

> Quality control samples inter-assay variations (n=25) in EIA Buffer

<table>
<thead>
<tr>
<th>CGRP (human)</th>
<th>C.V.</th>
<th>CGRP (human)</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.81 pg/mL</td>
<td>16.6 %</td>
<td>125 pg/mL</td>
<td>2.8 %</td>
</tr>
<tr>
<td>15.6 pg/mL</td>
<td>12.2 %</td>
<td>250 pg/mL</td>
<td>0.7 %</td>
</tr>
<tr>
<td>31.3 pg/mL</td>
<td>6.3 %</td>
<td>500 pg/mL</td>
<td>4.1 %</td>
</tr>
<tr>
<td>62.5 pg/mL</td>
<td>4.3 %</td>
<td>1000 pg/mL</td>
<td>-</td>
</tr>
</tbody>
</table>
**Cross-reactivity**

<table>
<thead>
<tr>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGRP-I/II (human)</td>
<td>100 %</td>
</tr>
<tr>
<td>CGRP-α/β (human)</td>
<td>100 %</td>
</tr>
<tr>
<td>CGRP-I/II (rat)</td>
<td>120 %</td>
</tr>
<tr>
<td>CGRP-α/β (rat)</td>
<td>120 %</td>
</tr>
<tr>
<td>CGRP (8-37)</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>Amylin</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>&lt;0.01 %</td>
</tr>
<tr>
<td>Substance P</td>
<td>&lt;0.01 %</td>
</tr>
</tbody>
</table>

**Specificity**

Comparison of HPLC profiles of a CGRP standard and a sample.
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 technique,
  - 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 you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Marketing Department by phone (+33 (0)139 306 260) or E-mail (marketing@bertinpharma.com).
1. Grassi J., Pradelles P.
Compounds labelled by the acetylcholinesterase of Electrophorus Electricus. Its preparation process and its use as a tracer or marquer in enzymo-immunological determinations.
*United States patent, N° 1,047,330. September 10, 1991*

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