GROWTH HORMONE (rat)

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For laboratory research use only. Not for human or veterinary diagnostic use.

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European patent # 89 139 552
U.S. patent # 50 47 330

Growth Hormone (rat)
Enzyme Immunoassay kit
#A05104.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#A11104
Version: 0117

Table of contents

96 wells
Storage: -20°C
Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Rabbit anti-goat precoated 96 well Strip Plate,	Blister with zip	A08104.1ea	1	-
Rat Growth Hormone Tracer	Green	A04104.100 dtn	1	Lyophilized
Rat Growth Hormone Antiserum	Red	A03104.100 dtn	1	Lyophilised
Rat Growth Hormone Standard	Blue with red septum	A06104.1 ea	2	Lyophilized
Rat Growth Hormone Quality Control	Green with red septum	A10104.1 ea	2	Lyophilized
Rat Growth Hormone EIA Buffer	Blue	A07104.1 ea	1	Lyophilized
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween 20	Transparent	A12000.1 ea	1	Liquid
Ellman's Reagent 50	Black with red septum	A09000_50.100 dtn	2	Lyophilized
Technical Booklet	-	A11104	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 33 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 QC samples provided in this kit have been prepared diluting rat plasma (Sprague Dawley rat) in EIA buffer. A sanitary control has been completed on Sprague Dawley rats following the Felasa Health Monitoring Recommendations. However, handle the CQ samples as a possible source of infection.

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.

Growth Hormone

Growth Hormone (GH) is a polypeptide hormone with a molecular weight of 23000 Daltons released from somatotropes of the anterior pituitary. It is regulated by several neurotransmitters and neuropeptides.

Among other functions it plays an essential role in regulating body growth ***[4, 5]***.

Principle of the assay

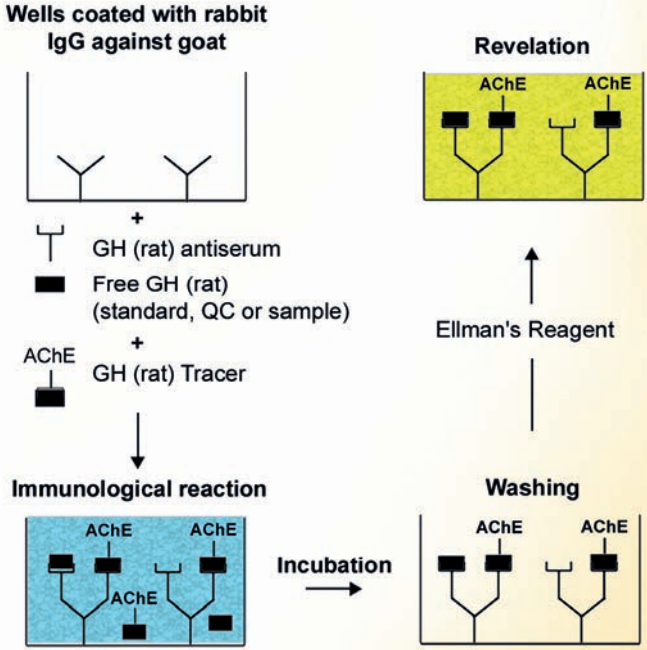
This Enzyme Immunometric Assay (EIA) is based on the competition between unlabelled (free) rat Growth Hormone (standards/QC/ samples) and acetylcholinesterase (AChE) linked to rat Growth Hormone (Tracer) for limited specific goat anti-rat GH antiserum sites.

The complex goat antiserum-rat GH (free GH or Tracer) binds to the rabbit polyclonal anti-goat immunoglobulin antibodies that are attached to the well.

The plate is washed to remove any unbound reagent, then Ellman's Reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

AChE acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm. The intensity of the colour, determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat GH present in the well during the immunological incubation.

The principle of the assay is summarised below:



Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water #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 collection and preparation

This assay may be used to measure rat GH in cell culture media or in plasma samples (see Bibliography hereafter for further information).

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.

Blood sample collection

Blood samples may be collected in tubes containing EDTA, lithium heparin, potassium oxalate or sodium citrate.

The samples are centrifuged at 1 600 g for 20 minutes. Plasma are collected and kept at -20°C until assay.

Blood sample preparation

No prior extraction procedure is necessary to measure GH in plasma samples and cell culture media.

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 33 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.

Growth Hormone (rat) EIA Buffer

Reconstitute the vial #A07104 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.

Growth Hormone (rat) Standard

Calibrated against the reference preparation NIDDK standard rGH-RP2

Reconstitute one Rat GH standard vial #A06104.1 ea with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of the first standard (S1) is 40 ng/mL.

Prepare seven polypropylene tubes (for the seven other standards S2 to S8) and add 500 µL of EIA buffer into each tube. Then prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of Assay Buffer	Standard concentration (ng / mL)
S1	-	-	40.00
S2	500 µL of S1	500 µL	20.00
S3	500 µL of S2	500 µL	10.00
S4	500 µL of S3	500 µL	5.00
S5	500 µL of S4	500 µL	2.50
S6	500 µL of S5	500 µL	1.25
S7	500 µL of S6	500 µL	0.63
S8	500 µL of S7	500 µL	0.31

Stability at 4°C:1 week.

▷ Growth Hormone (rat) Quality Control (on day 1)

Reconstitute one Quality Control vial #A10104 with 1 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 week.

▷ Growth Hormone (rat) Antiserum (on day 1)

Reconstitute the Antiserum vial #A03104 with 5 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 week.

▷ Wash Buffer (on day 1)

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL UltraPure water. Add 400 µL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content.
Stability at +4°C: 1 week.

Growth Hormone (rat) Tracer (on day 2)

Reconstitute the Tracer vial #A04104 with 5 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.

Ellman's Reagent (on day 3)

5 minutes before use (development of the plate), reconstitute one vial of Ellman's Reagent #A09000_50 with 50 mL of UltraPure water. 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

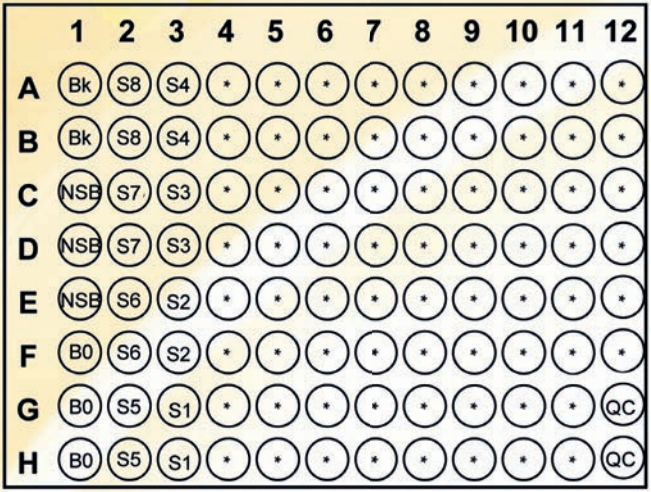
Prepare the wash buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.

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



Bk: Blank
B0: Maximum binding
*: Samples
NSB: Non Specific Binding
S8-S1 : Standards 8-1
QC: Quality Controls

▷ 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 and 50 µL to Maximum Binding (B0) wells.

> **Growth Hormone (rat) Standard**
Dispense 50 µL 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.

> **Growth Hormone (rat) Quality Control and Samples**
Dispense 50 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA Buffer.

> **Growth Hormone (rat) Antiserum**
Dispense 50 µL to each well, **except** Blank (Bk) wells and the Non Specific Binding (NSB) wells.

▷ Incubating the plate (first step)

Cover the plate with a cover sheet and incubate for 20 hours at room temperature.

▷ Distribution of tracer

Dispense 50 µL to each well **except** the Blank (B) wells.

Incubating the plate (second step)

Cover the plate with the cover sheet and incubate for 20 hours at room temperature.

Developing and reading the plate

- Reconstitute Ellman's Reagent as mentioned in the Reagent preparation section.
- Empty the plate by turning over. Rinse each well 5 times with 300 µL of Wash Buffer. 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 to each well. Cover the plate with an aluminum sheet and incubate in the dark at room temperature, on 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 (B0 wells) has reached a minimum of 0.2 A.U. (blank subtracted).

Enzyme Immunoassay Protocole (volumes are in µL)						
Volume / Wells	Blank	NSB	B0	Standard	QC	Sample
EIA Buffer	-	100	50	--	-	
Standard	-	-	-	50	-	-
QC					50	
Sample	-	-	-	-	-	50
Antiserum	-	-	50			
Cover plate, incubate 20 hours at +20°C						
Tracer	-	50				
Cover plate, incubate 20 hours at +20°C						
Wash strips 5 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, B0, standards, QC and samples.
- Calculate the B/B0 (%) for each standard, QC and sample: (average absorbance of standards, QC or sample - average absorbance of NSB) divided by (average absorbance of B0 - average absorbance of NSB) & multiplied by 100.
- Using a semi-log graph paper, plot the B/B0 (%) for each standard point (y axis) versus the concentration (x axis). Draw a best-fit line through the points.
- To determine the concentration of your samples, the corresponding B/B0 (%) value has to fall within the linear range of the standard curve (usual range of 20-80%). Find the B/B0 (%) value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Most plate readers are supplied with a curve-fitting software capable of graphing this type of data (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 (written on the label of the QC vial)

Acceptable range

- B0 absorbance: > 200 mAU blank subtracted in the conditions indicated above.
- NSB absorbance < 0.035.
- IC50 < 10 ng/mL.
- QC sample: ± 25% of the expected concentration (see the label on QC vial).

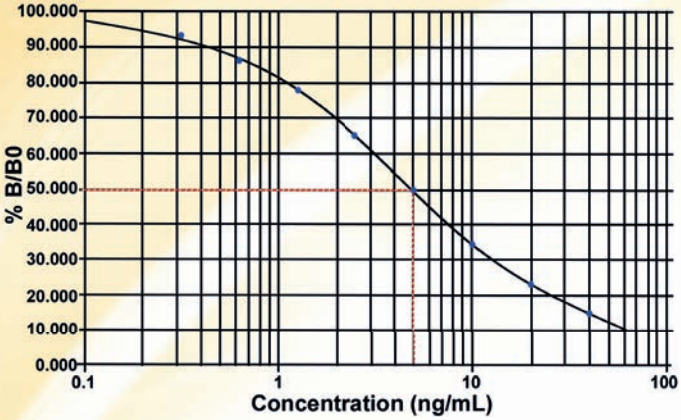
Typical results

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: 1 hour developing at +20°C, reading at 414 nm. A 4PL fitting was used to determine the concentrations.

	mAU	B/B0 (%)
NSB	3	-
B0	522	100
Standard 40 pg/mL	56	10.2
Standard 20 pg/mL	97	18.1
Standard 10 pg/mL	139	26.2
Standard 5 pg/mL	195	37.0
Standard 2.5 pg/mL	259	49.3
Standard 1.25 pg/mL	339	64.7
Standard 0.63 pg/mL	405	77.5
Standard 0.31 pg/mL	441	84.4
QC	241	-

Typical GH (rat) standard curve



Assay validation and characteristics

The Enzyme Immunometric assay of Growth Hormone (rat) has been validated by Ezan *et al*. for its use in rat plasma and cell culture media ***[6]***. Its main characteristics are the following.

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 producing 15 % displacement of initial tracer: 0.5 ng/mL.

- **Intra and inter-assay variations** are 4% (n = 24) and 14% (n=9)

- **Accuracy** in a hypophysectomised rat plasma sample

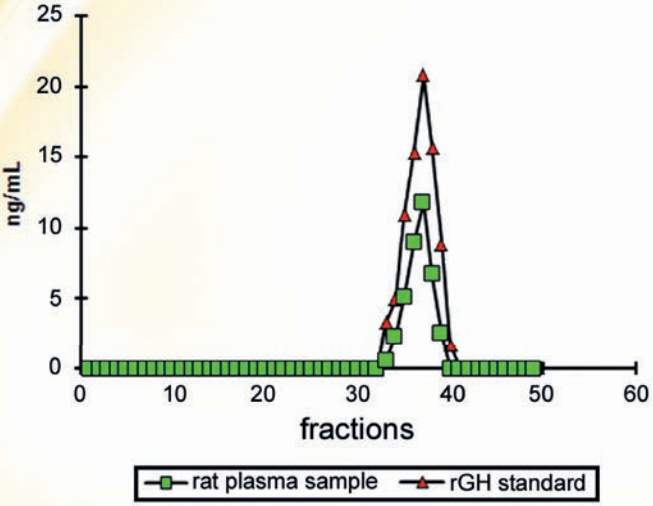
GH added	GH measured	Recovery
0 ng/mL	0 ng/mL	-
0.6 ng/mL	0.5 ng/mL	83 %
1.2 ng/mL	1.3 ng/mL	108 %
2.5 ng/mL	2.5 ng/mL	100 %
5 ng/mL	5 ng/mL	100 %
10 ng/mL	10.1 ng/mL	101 %
20 ng/mL	24.4 ng/mL	122 %

> Cross-reactivity

Compound	%	Compound	%
Mouse GH	91 %	Human GH	<0.1 %
Rat prolactin	<1 %	Rat TSH	<0.1 %
Rat FSH	<0.1 %	Rat LH	<0.1 %

> Specificity

Comparison of HPLC profiles of Growth Hormone (rat) standard and a plasma 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.

> **Analyses of two dilutions of a biological sample do not agree**: Interfering substances are present. Sample must be purified prior to EIA analysis (excepting plasma samples).

> **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).

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3. Pradelles P., Grassi J. and Maclouf J.
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Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies.
J Pharm Biomed Anal. (2011) 55(5) : 869-877

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Guideline on bioanalytical method validation, 21 July 2011

Additional readings

List of publications quoting the use of this kit

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Overexpression of Gly56/Gly80/Gly81-Mutant Insulin-Like Growth Factor-Binding Protein-3 in Transgenic Mice.
Endocrinology, March 2005, 146(3):1523–1531

12. Ma J.N., Schiffer H.H., Knapp A.E. *et al.*
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Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and bio-chemicals thanks to its innovative work in research and development.
Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

Bertin Pharma is active worldwide either with direct sales or through our qualified and trained international distribution network from the United States to Japan.

We are able to provide you with local technical support to use at ease our products.

For further information, please send your request to
bioreagent@bertinpharma.com

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Parc d'activités du Pas du Lac - 10 bis avenue Ampère
F-78180 Montigny-le-Bretonneux - France
Tel: +33 (0)139 306 036 - Fax: +33 (0)139 306 299
bioreagent@bertinpharma.com - www.bertinpharma.com