



## 20-HYDROXYECDYSONE

A brand name of

**bertin**  
pharma

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

**Bertin Pharma also markets pre-analytical products, EIA kits, antibodies, CYP450s & biochemicals for:**

- ▶ **Cardiology / Hypertension**
- ▶ **Diabetes / Obesity**
- ▶ **Endocrinology / Metabolism**
- ▶ **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](mailto:bioreagent@bertinpharma.com)**



European patent # 89 139 552

U.S. patent # 50 47 330

**20-Hydroxyecdysone  
Enzyme Immunoassay kit  
#A05120.96 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

#A11120  
Version: 0115

## Table of contents

▶ Precaution for use	6
▶ Background	7
▶ Principle of the assay	10
▶ Materials and equipment required	12
▶ Sample collection and preparation	13
▶ Reagent preparation	14
▶ Assay procedure	17
▶ Data analysis	22
▶ Acceptable range	24
▶ Typical results	25
▶ Assay validation and characteristics	26
▶ Troubleshooting	27
▶ Bibliography	28

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

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Mouse anti-Rabbit precoated 96-well Strip Plate	blister with zip	A08100.1 ea	1	-
20-Hydroxyecdysone Tracer	green	A04120.100 dtn	1	Lyophilized
20-Hydroxyecdysone Antiserum	red	A03120.100 dtn	1	Lyophilized
20-Hydroxyecdysone Standard	blue with red septum	A06120.1 ea	2	Lyophilized
20-Hydroxyecdysone Quality Control	green with red septum	A10120.1 ea	2	Lyophilized
EIA Buffer	blue	A07000.1 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
Instruction booklet	-	A11120.1 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 34 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 the 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 it's 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<sup>-1</sup>. That is nearly 3 times faster than Horse Radish 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<sup>®</sup> 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<sup>®</sup> is a completely stable enzyme, unlike peroxidase which is suicidal. Thus, if a plate is accidentally dropped after dispatch of the AChE<sup>®</sup> substrate (Ellman's reagent), 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 while waiting for technical advice from the Bioreagent Department.

## ▷ **20-Hydroxyecdysone**

Ecdysone is a steroid hormone of the ecdysteroids family which is found in animals (vertebrates and invertebrates) and in plants. There exist different polyhydroxylated forms of ecdysone, the major one being the 20-Hydroxyecdysone.

20-Hydroxyecdysone (20E) is known as the insect moulting hormone **[20]**. Indeed, in interaction with the juvenile hormone (JH), 20E plays a key role in growth, development, metamorphosis and reproduction **[4, 5]** of the Arthropods (i.e. invertebrates such as insects, crustaceans, arachnids, etc.) **[6, 7, 8]**.

20E regulates target gene transcription by binding a heterodimeric nuclear receptor composed of the ecdysone receptor (EcR) and Ultraspiracle (USP) **[9, 10, 11]**.

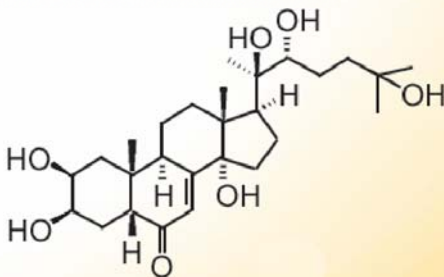


Various plants synthesize plant-derived ecdysteroids. However, the role of these phytoecdysteroids (PEs) is not currently well-known. 20E is widespread in plants and represents the main PE. These hormones may have a potential role in plant defense against herbivore pest insects and nematodes [12, 13].

Little is known about the function of ecdysteroids in the vertebrates. There are several reports which indicate an effect of the phycoecdysteroid 20E on muscle growth and fat loss in mammals. Some dietary supplements are marketed to enhance physical performances of athletes, and particularly of bodybuilders. Besides, researchers are currently studying the effect of 20E in the treatment of obesity and diabetes [14, 15]. And there are on-going investigations on the possible interest of 20E in the prevention and the treatment of diseases such as osteoporosis and osteoarthritis [16, 17].

**Synonyms:** 20E, insect molting hormone, 20-OH ecdysone,  $\beta$ -ecdysone,  $\beta$ -ecdysterone, crustecdysterone, ecdysterone, 20- $\beta$ -hydroxyecdysterone.

### 20-Hydroxyecdysone Structure



## ▶ Principle of the assay

This Enzyme Immunoassay (EIA) is based on the competition between unlabelled 20-Hydroxyecdysone and acetylcholinesterase (AChE®)-labelled 20-Hydroxyecdysone (Tracer) for limited specific rabbit anti-20-hydroxyecdysone antiserum sites.

The complex rabbit antiserum – 20-Hydroxyecdysone (free 20E or Tracer) binds to the mouse monoclonal anti-rabbit antibody coated in the well.

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

The AChE tracer acts on the Ellman's Reagent to form a yellow compound that strongly absorbs at 414 nm.

The intensity of the color, determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free 20E present in the well during the immunological incubation.

The kit has been validated by Bertin Pharma in EIA buffer, and has not been tested on biological samples. However, several scientists used immunoassay technics to quantify 20-hydroxyecdysone in biological samples like haemolymph **[18, 20, 21, 22]**.

The principle of the assay is summarised below:

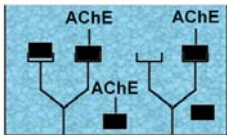
**Wells coated with mouse IgG against rabbit**



+

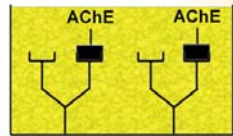


**Immunological reaction**

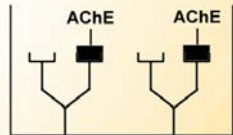


**Washing**

**Revelation**



↑  
Ellman's reagent



## ► **Materials and equipment required**

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

- > Precision micropipettes (20 to 1000  $\mu\text{L}$ )
- > Multichannel pipette 100  $\mu\text{L}$  or 200  $\mu\text{L}$  and disposable tips
- > Spectrophotometer plate reader (405 or 414 nm filter)
- > Microplate washer (or washbottles)
- > Microplate shaker
- > Magnetic stirring bar
- > UltraPure water
- > Polypropylene tubes



Water used to prepare all EIA reagents and buffers must be UltraPure, deionized & free from organic contaminants traces.

Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer AcetylCholinesterase. 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**

The kit has been validated in EIA buffer, and has not been tested on biological samples. However, several scientists used immunoassay technics to quantify 20-hydroxyecdysone in biological samples like haemolymph **[18, 20, 21, 22]**.

It is the responsibility of the user to find the proper dilution according to the type of biological material and to check for potential interferences (see our website or contact our technical support).

We suggest the following protocol to prepare biological samples **[18, 19]** :

- Extraction of the 20-Hydroxyecdysone (which is hydrophobic) with methanol
- Centrifugation and collection of the supernatant
- Evaporation until completely dry
- Dilution in EIA buffer

## ▶ Reagent preparation

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*

### ▶ 20-hydroxyecdysone Standard

Reconstitute the 20-hydroxyecdysone Standard vial #A06120 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 this first standard **S1** is 5000 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:

Standard	Volume of Standard	Volume of Assay Buffer	Standard concentration
S1	-	-	5 000 pg/mL
S2	500 µL of S1	500 µL	2 500 pg/mL
S3	500 µL of S2	500 µL	1 250 pg/mL
S4	500 µL of S3	500 µL	625 pg/mL
S5	500 µL of S4	500 µL	312.5 pg/mL
S6	500 µL of S5	500 µL	156.3 pg/mL
S7	500 µL of S6	500 µL	78.1 pg/mL
S8	500 µL of S7	500 µL	39.1 pg/mL

*Stability at 4°C: 1 week*

### ▷ 20-hydroxyecdysone Quality Control

Reconstitute the vial #A10120 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: 1 week*

### ▷ 20-hydroxyecdysone Tracer

Reconstitute the vial #A04120 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*

### ▷ **20-hydroxyecdysone Antiserum**

Reconstitute the vial #A03120 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**

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL of UltraPure water. Add 400 µL of Tween20 #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\_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 assay 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, store at +4°C for 1 month maximum.

Rinse each well 4 times with the Wash Buffer 300  $\mu$ L/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.

### ▶ **Distribution of reagents and samples**

A plate set-up is suggested on the following page. The content 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 pipette the buffer, standard, sample, 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  $\mu\text{L}$  to NSB wells and 50  $\mu\text{L}$  to B0 wells.

> **20-hydroxyecdysone Standards**

Dispense 50  $\mu\text{L}$  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.

> **Quality Control and samples**

Dispense 50  $\mu\text{L}$  in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

> **20-hydroxyecdysone Tracer**

Dispense 50  $\mu\text{L}$  to each well, **except** Blank (Bk) wells.

> **20-hydroxyecdysone Antiserum**

Dispense 50  $\mu\text{L}$  to each well **except** Blank (Bk) wells and Non Specific Binding (NSB) wells.

> **Incubating the plate**

Cover the plate with the cover sheet and incubate overnight at  $+4^{\circ}\text{C}$ .



## ▷ **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  $\mu$ L 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 200 $\mu$ L of Ellman's reagent to each 96 well. Cover the plate with an aluminium foil sheet and incubate in the dark at room temperature. Optimal development is obtained using an orbital shaker.
- ▷ Gently 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 0.200 A.U. blank subtracted.**

<b>Enzyme Immunoassay Protocole (volumes are in <math>\mu\text{L}</math>)</b>						
	Blank	NSB	B0	Standard	QC	Sample
EIA Buffer	-	100	50	-	-	-
Standard	-	-	-	50	-	-
QC	-	-	-	-	50	-
Sample	-	-	-	-	-	50
Tracer	-	50				
Antiserum	-	-	50			
Cover plate, incubate overnight at +4°C						
Wash plate 5 times & discard liquid from the wells						
Ellman's reagent	200					
Incubate with an orbital shaker at 400 rpm 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 wells (absorbance of Ellman's reagent alone) from the absorbance readings of the rest of the plate. If not, do it now.

- Subtract the average absorbance of NSB for each B<sub>0</sub>, standards, quality control and samples.
- Calculate the average absorbance for each B<sub>0</sub>, standard, quality control and sample.
- Calculate the B/B<sub>0</sub> (%) for each standard, QC and sample (average absorbance of standards, QC or sample divided by average absorbance of B<sub>0</sub>) & multiplied by 100.
- Using a semi-log graph paper for each standard point, plot the B/B<sub>0</sub> (%) on y axis versus the concentration (pg/mL) on x axis. Draw a best-fit line through the points.
- To determine the concentration of your sample, the corresponding B/B<sub>0</sub> (%) value has to be comprised between 20% and 80%. Find the B/B<sub>0</sub> (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Diluted samples which concentration determined on standard curve is greater than 5000 pg/mL should be re-assayed after appropriated dilution in EIA buffer.

- > Most plate readers are supplied with curve-fitting software capable of graphing these data (4-parameter or 5-parameter logistic fit). 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 < 35 mAU
- ▶ IC50: <1000 pg/mL
- ▶ QC sample:  $\pm 25\%$  of the expected concentration (see the label of QC vial).



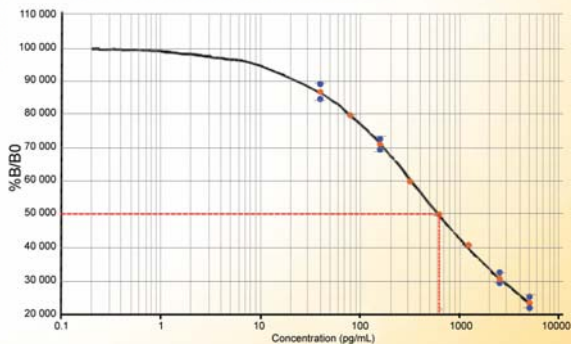
## ► Typical results

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

	20-Hydroxyecdysone pg/mL	mAU	B/B0 (%)
Standard S1	5000	120	23.2
Standard S2	2500	160	31.0
Standard S3	1250	211	40.8
Standard S4	625	256	49.5
Standard S5	312.5	310	59.9
Standard S6	156.3	369	71.3
Standard S7	78.1	413	79.7
Standard S8	39.1	451	87.1
B0	0.0	518	100.0

Typical 20-hydroxyecdysone standard curve



## ► Assay validation and characteristics

The Enzyme Immunoassay of 20-Hydroxyecdysone has been validated in EIA Buffer only.

- > The **limit of detection (LOD)** of 20-Hydroxyecdysone corresponding to the B0 average minus three standard deviations is around 31 pg/mL.
- > The **IC50** is the concentration in 20-Hydroxyecdysone corresponding to 50 % of the maximum Binding is less than 1000 pg/mL.
- > **Cross-reactivity [18]**

20-hydroxy-ecdysone	100%
Ecdysone	100%
2-deoxy-20-hydroxy-ecdysone	88%
Polypodine B	70%
2-deoxy-ecdysone	63%
Ponasterone A	43%
Cyasterone	5%
Podecdysone C	4.5%
Makisterone A	4%
26-hydroxy-ecdysone	1.4%
Muristerone A	1.2%
Kaladasterone	1%
22-epi-ecdysone	< 0.1%
Posterone	< 0.1%

## ► Troubleshooting

- > **Absorbance values are too low:** organic contamination of water, incubation in wrong conditions (time or temperature), reading time not long enough, 20-Hydroxyecdysone Tracer has not been dispensed.
- > **High signal and background in all wells:** inefficient washing or overdeveloping (incubation time should be reduced) or high ambient temperature.
- > **High dispersion of duplicates:** poor pipetting technique or irregular plate washing.

These are a few examples of trouble shooting 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](mailto:bioreagent@bertinpharma.com)), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005.2x96 wells and EIA workshop upon request. For further information, please contact our Marketing Dpt by phone (+33 (0)139 306 260) or E-mail ([marketing@bertinpharma.com](mailto:marketing@bertinpharma.com)).

## ► Bibliography

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 enzyme-immunological determinations.  
*United States patent, N° 1,047,330. September 10, 1991*
2. Grassi J, Pradelles P,  
The use of Acetylcholinesterase as a Universal marker in Enzyme-Immunoassays  
*Proceedings of the Third International Meeting on Cholinesterases, American Chemical Society (1991)*
3. Pradelles P, Grassi J, Maclouf J.  
Enzyme Immunoassays of Eicosanoids Using Acetylcholinesterase  
*Methods in Enzymology (1990), vol. 187, 24-34*  
Mol Aspects Med. 2003 Aug-Oct;24(4-5):281-91. Review.
4. A. Abrieux, L. Duportets et al  
The GPCR membrane receptor, DopEcR, mediates the actions of both dopamine and ecdysone to control sex pheromone perception in an insect.  
*Frontiers in Behavioral Neuroscience, vol.8, article 312, September 2014*
5. P. Gabrieli, E. Kakani *et al*  
Sexual transfer of the steroid hormone 20E induces the postmating switch in *Anopheles gambiae*.  
*PNAS, vol. 111, No.46, p. 16353-16358, November 18, 2014*

- 6.** D. Muramatsu, T. Kinjoh, T. Shinoda and K. Hiruma  
The role of 20-hydroxyecdysone and juvenile hormone in pupal commitment of the epidermis of the silkworm, *Bombyx mori*.  
*Mechanisms of development* 125, 411-420, 2008
- 7.** M. Iga and H. Kataoka  
Recent studies on insect hormone metabolic pathways mediated by cytochrome P450 enzymes.  
*Biological and Pharmaceutical Bulletin*, 35(6) 838-843, 2012
- 8.** P-C. Liu, J-X. Wang, Q-S. Song and X-F. Zhao  
The participation of calponin in the cross talk between 20-Hydroxyecdysone and juvenile hormone signaling pathways by phosphorylation variation.  
*PLOS One*, vol.6, No.5, e19776, May 2011
- 9.** A. Tan and S. R. Palli  
Ecdysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red flour beetle, *Tribolium castaneum*.  
*Molecular and Cellular Endocrinology*, 291(1-2): 42-49, September 10, 2008
- 10.** C. Browning, E. Martin *et al*  
Critical role of desolvation in the binding of 20-Hydroxyecdysone to the Ecdysone receptor.  
*The Journal of Biological Chemistry*, vol.282, No.45, p.32924-32934, November 9, 2007

- 11.** R. B. Beckstead, G. Lam and C. Thummel  
The genomic response to 20-hydroxyecdysone at the onset of *Drosophila metamorphosis*.  
*Genome Biology*, 6:R99, November 2005
- 12.** J-Q. Zhu, S. Liu *et al*  
Improvement of pest resistance in transgenic tobacco plants expressing dsRNA of an insect-associated gene EcR.  
*PLOS One*, vol.7, No.6, e38572, June 2012
- 13.** K. H. Boo, D. Lee *et al*  
Distribution and biosynthesis of 20-Hydroxyecdysone in plants of *Achyranthes japonica* Nakai.  
*Bioscience, Biotechnology and Biochemistry*, 74 (11), 2226-2231, 2010
- 14.** D. Cheng, L. Kutzler *et al*  
Continuous infusion of 20-Hydroxyecdysone increased mass of triceps brachii in C57BL/6 mice.  
*Phytotherapy Research*, 27(1): 107-111, January 2013
- 15.** P. Kizelsztejn, D. Govorko *et al*  
20-Hydroxyecdysone decreases weight and hyperglycemia in a diet-induced obesity mice model.  
*American Journal of Physiology-Endocrinology and Metabolism*, 296: E433-E439, 2009
- 16.** L. Gao, G. Cai and X. Shi  
β-ecdysone induces osteogenic differentiation in mouse mesenchymal stem cells and relieves osteoporosis.  
*Biological and Pharmaceutical Bulletin*, 31(12) 2245-2249, 2008

- 17.** S-Y. Sheu, S-R. Ho *et al*  
Arthropod steroid hormone (20-Hydroxyecdysone) suppresses il-1 $\beta$ -induced catabolic gene expression in cartilage.  
*BMC Complementary and Alternative Medecine*, 15: 1, 2015
- 18.** P. Porcheron, M. Morinière, J. Grassi and P. Pradelles  
Development of an enzyme immunoassay for ecdysteroids using acetylcholinesterase as label.  
*Insect Biochemistry*, vol. 19, No.2, p.117-122, 1989
- 19.** N. Pascual, X. Bellés *et al*  
Quantification of ecdysteroids by immunoassay : comparison of enzyme immunoassay and radioimmunoassay.  
*Zeitschrift für Naturforschung*, 50c, 862-867, 1995
- 20.** J. Claret, P. Porcheron and F. Dray  
Critical period and ecdydione titers in the pupae of *Pieris brassicae*.  
*Separatum Experientia* 33, 1389, 1977
- 21.** P. Porcheron, J. Foucrier, Cl. Gros, P. Pradelles, P. Cassier and F. Dray  
Radioimmunoassay of arthropod moulting hormone:  $\beta$ -ecdysone antibodies production and  $^{125}\text{I}$ -Iodinated tracer preparation.  
*FEBS Letters*, vol. 61, No.2, p.159, 1976
- 22.** P. Porcheron, P. Cassier and F. Dray  
Applications de techniques immunochimiques à l'étude de l'ecdysone.

*Bull. Soc. Zool. France, Suppl. 1, p71-75, 1977*

*This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine readable form without the prior consent, in writing, from Bertin Pharma.*







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](mailto:bioreagent@bertinpharma.com)



care. innovate. deliver.

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](mailto:bioreagent@bertinpharma.com) - [www.bertinpharma.com](http://www.bertinpharma.com)

— A company of Group **ENIM** —