INSULIN (mouse, rat)

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 & biochemicals for:

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Do not hesitate to contact our after-sales services for further information at bioreagent@bertinpharma.com

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

Insulin (mouse, rat)
Enzyme Immunoassay kit
#A05105.96 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

#A11105
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
Goat anti-Guinea pig pre-coated 96-well Strip Plate	Blister with zip	A08105.1 ea	1	-
Insulin (mouse, rat) Tracer	Green	A04105.100 dtn	1	Lyophilised
Insulin (mouse, rat) Anti-serum	Blue with red septum	A03105.100 dtn	1	Lyophilised
Insulin (mouse, rat) Standard	Green with red septum	A06105.1 ea	2	Lyophilised
EIA Buffer	Blue	A07000.1 ea	1	Lyophilised
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween 20	Transparent	A12000.1 ea	1	Liquid
Insulin (mouse, rat) Quality Control	Black with red septum	A10105.1 ea	2	Lyophilised
Ellman's Reagent 50	Black with septum	A09000_50.100 dtn	2	Lyophilised
Technical Booklet	-	#A11105.1 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 35 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 by diluting rat plasma (Sprague Dawley rat) in EIA uffer. A sanitary control has been completed on Sprague Dawley rats following the Felasa Health Monitoring Recommendations. However, handle the QC 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.

Principle of the assay

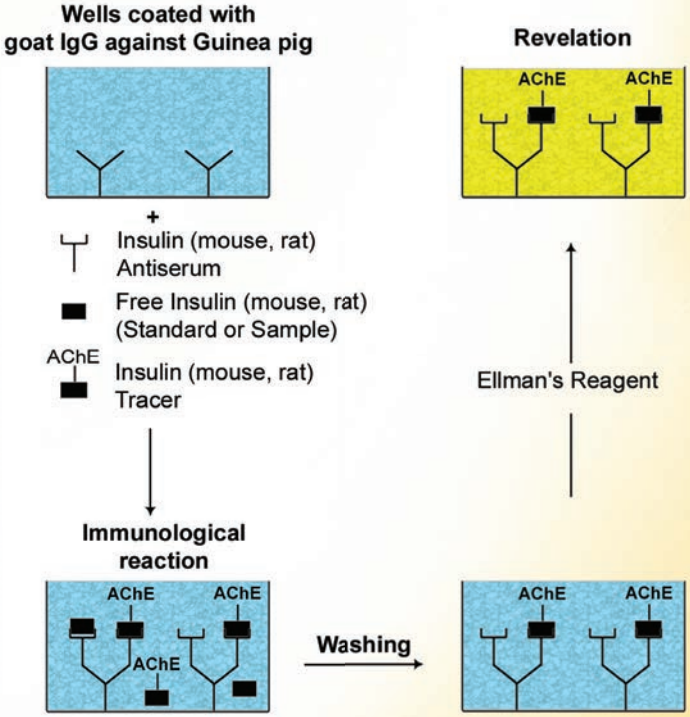
This Enzyme Immunometric Assay (EIA) is based on the competition between unlabelled rat insulin and acetylcholinesterase (AChE) linked to rat insulin (tracer) for limited specific Guinea-Pig anti-rat insulin antiserum sites.

The complex Guinea-Pig antiserum-rat insulin (free insulin or tracer) binds to the Goat anti-Guinea-Pig antibody that is attached to the well.

The plate is then washed 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.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of tracer bound to the well and is inversely proportional to the amount of free rat insulin 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:

For the assay:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or washbottles)
- Orbitral microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water (item number #A07001.1L)
- Polypropylene tubes

Water used to prepare all EIA reagents and buffers must be Ultra Pure, 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

This assay may be used to measure insulin in mouse and rat plasma or serum sample. To do so, blood samples are collected in tubes containing heparin or EDTA. The samples are centrifuged at 1 600 g for 20 minutes.

Plasmas are collected and kept at -20°C until assay.

No prior extraction procedure is necessary to measure insulin in plasma samples. However, hemolysis interferes with the assay by degrading insulin. SPI-Bio has developed an inhibitor cocktail and a procedure presented hereafter to prevent hemolysis consequences. Users are recommended to follow it in such a case.

Inhibitor Cocktail

Dilute 226.1 mg of tetrahydrated sodium salt in 500 µL of distilled or deionized water and 65 mg of phenanthroline monohydrate in 500 µL of methanol.
Mix them together.

Inhibitor Buffer

Dilute 250 µL of the inhibitor cocktail in 25 mL of EIA Buffer provided in the kit.

Afterwards, prepare Insulin (mouse, rat) Standards, Quality Control and Samples as follows:

Insulin (mouse, rat) Standard

Reconstitute the Standard vial (item #A06105.1 ea) with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. Then, add 10 µL of the inhibitor cocktail.
Prepare seven propylene tubes for the other standards and add 500 µL of the Inhibitor Buffer into each tube.
Add 500 µL of the first tube (containing the first standard) to the second tube.
Continue this procedure for the other tubes.

Quality Control

Reconstitute one vial with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then add 10 µL of the Inhibitor Cocktail and then mix thoroughly by gentle inversion.

Sample

Prior dispatching, add 10 µL of Inhibitor Cocktail for 1 mL of plasma (or 5 µL for 500 µL, 2 µL for 200 µL, etc.). If necessary, dilute the sample with the Inhibitor Cocktail Buffer.

If no hemolysis is observed in plasma sample, prepare the above-mentioned reagents as indicated in the next section: Reagent 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.

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 35 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.

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

Insulin (mouse, rat) Standard

Reconstitute the Standard vial (item #A06105.1 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 10 ng/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	-	-	10 ng/mL
S2	500 µL of S1	500 µL	5 ng/mL
S3	500 µL of S2	500 µL	2.5 ng/mL
S4	500 µL of S3	500 µL	1.25 ng/mL
S5	500 µL of S4	500 µL	0.63 ng/mL
S6	500 µL of S5	500 µL	0.31 ng/mL
S7	500 µL of S6	500 µL	0.16 ng/mL
S8	500 µL of S7	500 µL	0.08 ng/mL

Stability at 4°C: 24 hours

▷ Quality Control

Reconstitute one vial 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: 24 hours.

▷ Insulin (mouse, rat) Tracer

Reconstitute one vial 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.

Insulin (mouse, rat) Antiserum

Reconstitute one vial 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.

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_50.100 dtn with 50 mL of UltraPure water. The tube content should be thoroughly mixed.
Stability at +4°C and in the dark: 1 day.

Assay procedure

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

Plate preparation

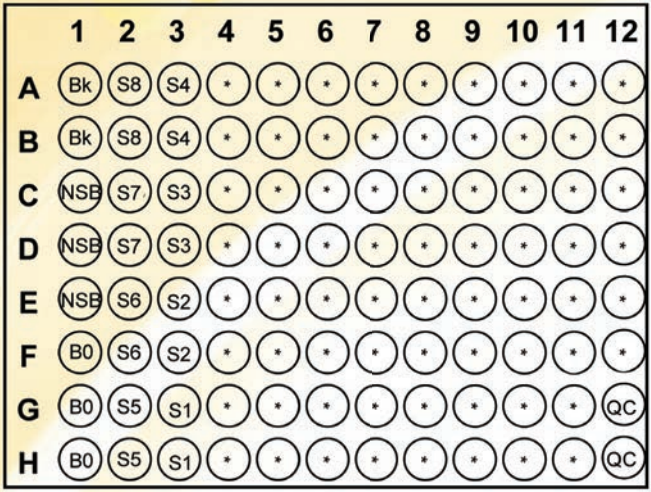
Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.

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.



Bk : Blank

NSB : Non Specific Binding

* / QC : Samples or Quality Controls

B0: Maximum Binding

S1-S8 : Standards 1-8

▷ 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 expeling with the pipette tip.

> **EIA Buffer**

Dispense 100 µL to Non Specific Binding (NSB) wells and 50 µL to Maximum Binding (B0) wells.

> **Insulin (mouse, rat) Standard**

Dispense 50 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.

> **Quality Control and samples**

Dispense 50 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA Buffer.

> **Insulin (mouse, rat) Tracer**

Dispense 50 µL to each well, **except** Blank (Bk).

> **Insulin (mouse, rat) Antiserum**

Dispense 50 µL to each well, **except** Blank (Bk) wells and Non Specific Binding (NSB) wells.

▷ Incubating the plate

Cover the plate with a cover sheet and incubate for 16-20 hours at 4°C (optimal temperature).

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. 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 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 (B0 wells) has reached 0.2-0.8 A.U. (blank subtracted).

Enzyme Immunoassay Protocol (volumes are in µL)						
Volume / Wells	Blank	NSB	B0	Standard	QC	Sample
Buffer	-	100	50	-		
Standard	-			50	-	
Sample	-				50	
Tracer	-	50				
Antiserum	-		50			
Cover plate, incubate 16-20 hours at +4°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 wells (absorbance of Ellman's Reagent alone) from the absorbance readings of the rest of the plate. If it is not the case, please do it.

- Calculate the average absorbance for each NSB, B0, standard and samples.
- For each standard and sample, calculate the B/B0 (%) 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 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.
- Samples with a concentration greater than 10 ng/mL should be re-assayed after dilution in EIA Buffer.
- Most plate readers are supplied with a 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

- B0 absorbance > 200 mAU in the conditions indicated above.
- Ratio NSB absorbance / B0 absorbance: < 0.1 and NSB < 35 mAU.
- 50% B/B0 (%): < 1.6 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.

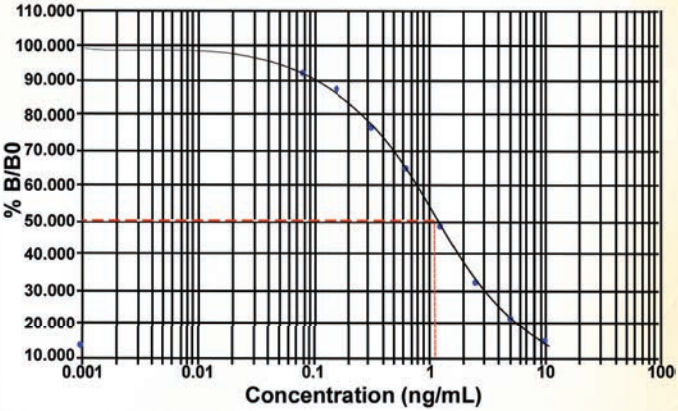
These data were obtained using all reagents as supplied in this kit under the following conditions: 90 minutes developing at +20°C, reading at 414 nm. A logit/log curve fitting was used to determine the concentrations.

	mAU	B/B0 (%)
NSB	3	-
B0	453	100
Standard 10 ng/mL	64	13.6
Standard 5 ng/mL	90	19.3
Standard 2.50 ng/mL	126	27.3
Standard 1.25 ng/mL	171	37.3
Standard 0.63 ng/mL	235	51.6
Standard 0.31 ng/mL	293	64.4
Standard 0.16 ng/mL	352	77.6
Standard 0.08 ng/mL	384	84.7

> Cross-reactivity

Insulin (rat)	100 %
Insulin (hamster)	100 %
Insulin (human)	100 %
Insulin (mouse)	100 %
Insulin (pig)	100 %
Insulin (sheep)	100 %

Typical Insulin (mouse, rat) standard curve



Troubleshooting

> **B0 value is too low**:
- incubation in wrong conditions (time or temperature),
- reading time too short,
- Insulin (mouse, rat)-AChE Tracer, Insulin (mouse, rat) Antiserum or Ellman's Reagent have not been dispensed.

> **NSB value too high:**
- contamination of NSB wells with Insulin (mouse, rat) Antiserum
- inefficient washing.

> **High dispersion of duplicates**:
- poor pipetting technique,
- irregular plate washing.

> **IC50 or QC concentrations not within the expected range**:
- wrong preparation of standards.

> **Analyses of two dilutions of a biological sample do not agree**:
- Interfering substances are present. Sample must be purified prior to EIA analysis (except 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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Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and biochemicals 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.

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bioreagent@bertinpharma.com

bertin pharma

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