bertin bioreagent

Insulin (human)

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

Bertin Bioreagent also markets pre-analytical products, EIA kits, antibodies & biochemicals for:

- **Inflammation**
- **Oxidative injury**
- **Endocrinology**
- **Diabetes**
- **Obesity**
- **Hypertension**
- **Pain**
- **Prion diseases**

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

Insulin (human)
ELISA kit
#A05322.96 wells

For research laboratory use only
Not for human diagnostic use

This assay was developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11322
Version: 0124

Table of contents

96 wells

Storage: +4°C

Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Insulin precoated 96-well Strip Plate	Blister with zip	A08322.1 ea	1	-
Insulin (human) Tracer	Green	A04322.100 dtn	1	Lyophilised
Insulin (human) Standard	Blue with red septum	A06322.1 ea	2	Lyophilised
Insulin (human) Quality Control	Green with red septum	A10322.1ea	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
Ellman's reagent 49+1	Black	A09000_49+1.100 dtn	2	Lyophilised
Technical Booklet	-	A11322.1 ea	1	-
Well cover Sheet	-	-	1	-

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

Precaution for use

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

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

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

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

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

Temperature

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

Background

Acetylcholinesterase AChE Technology

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

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

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

substrate of enzymes that are inherently unstable.

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

Insulin

Insulin is a polypeptide composed of 51 amino acids divided into two chains (A and B) (4) linked by-disulfide bond. Both chains are organized as helices alpha.

Insulin is produced by the beta cells of the pancreatic islets in form of a precursor (prepro-insulin) and is modified to obtain the pro-insulin (peptide signal elimination) and finally insulin (peptide C elimination).

The main function of the insulin is the regulation of the glucose concentration in the organism.

The dysregulation of the production of insulin induced many disorders including diabetes.

Principle of the assay

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

Insulin introduced into the wells (standard or sample) is bound by the monoclonal antibody coated on the plate and is then detected by another monoclonal antibody tagged with acetylcholinesterase (AChE) also specific of insulin.

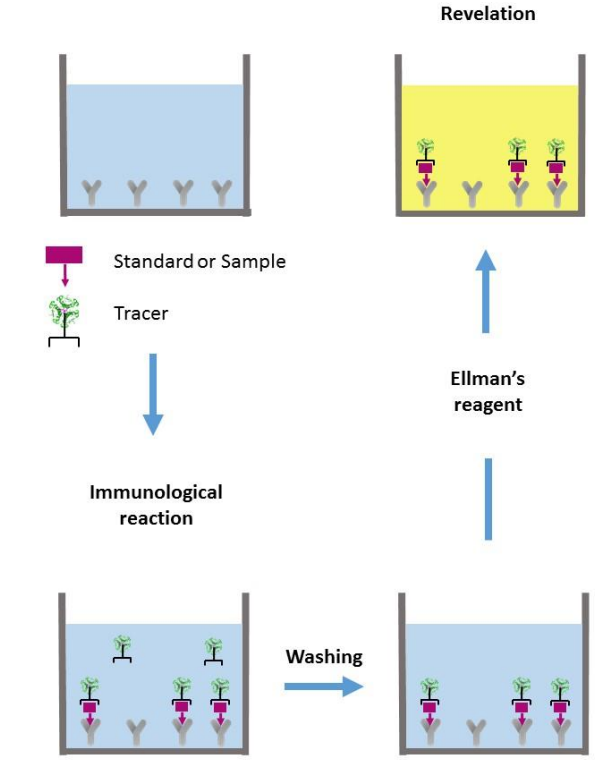
The two antibodies then form a sandwich by binding on different epitopes of the insulin.

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

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

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

The principle of the assay is summarised below:



Assay characteristics

- ***Validated for human samples***

- ***Limit of detection (LOD):*** ≤0.3 µUI/mL (calculated as the concentration of insulin corresponding to the NSB average plus three standard deviations)

- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
Insulin (pig)	100 %
Pro-Insulin (human)	Not detected
C-Peptide (human)	Not detected

- ***Assay validation data:*** ask Bertin Bioreagent (tech@bertin-bioreagent.com) or your local distributor for a copy of the validation data with human samples.

Materials and equipment required

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

For the assay:

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

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Do not use distilled water, HPLC-grade water or sterile water.

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

Sample collection and preparation

This assay has been validated to measure insulin in buffer and in plasma sampled on EDTA K3

General precautions

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

Sample collection

Blood samples are collected in tubes containing EDTA-K3.

Hemolysed plasma must be excluded of the study.
To prevent degradation of the insulin by insulinase, an inhibitor cocktail (D05011) could be added in to the sample during collection time. Contact the technical support.

Sample preparation

Plasma samples may be assayed directly without any extraction procedure after being **diluted at least to 1:10 in EIA Buffer** (50µL of plasma + 450µL of EIA buffer) in order to avoid the matrix effect.

Reagent preparation

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

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

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

EIA Buffer

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

Stability at 4°C: 1 month.

Insulin (human) Standard

Reconstitute the Insulin Standard vial #A06322 with 1 mL of UltraPure water. Allow standard to stand for 5 minutes or until it is completely dissolved. Mix standard thoroughly by gentle inversions.

The concentration of the first standard (S1) is 52 µUI/mL. Prepare seven polypropylene tubes (for the seven other standards) and add 500 µL of EIA Buffer into each tube. Then

prepare the standards by serial dilutions as indicated in following table. Mix each tube thoroughly before the next transfer.

Standard	Volume of Standard	Volume of EIA Buffer	Standard concentration
S1	-	-	52.0 µUI/mL
S2	500 µL of S1	500 µL	26.0 µUI/mL
S3	500 µL of S2	500 µL	13.0 µUI/mL
S4	500 µL of S3	500 µL	6.5 µUI/mL
S5	500 µL of S4	500 µL	3.3 µUI/mL
S6	500 µL of S5	500 µL	1.6 µUI/mL
S7	500 µL of S6	500 µL	0.8 µUI/mL
S8	500 µL of S7	500 µL	0.4 µUI/mL

Stability at 4°C: within the day

Insulin (human) Quality Control

Reconstitute the Insulin QC vial #A10322 with 1 mL of UltraPure water. Allow quality control to stand for 5 minutes or until it is completely dissolved. Mix quality control thoroughly by gentle inversions.

Stability at 4°C: within the day.

Insulin (human) Tracer

Reconstitute the Insulin Tracer vial #A04322 with 5 mL of EIA Buffer. Allow tracer to stand for 5 minutes or until

it is completely dissolved. Mix tracer thoroughly by gentle inversions.

Stability at +4°C: 2 weeks.

Wash Buffer

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL of UltraPure water. Add 400 µL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content. Note that concentrated wash buffer is also used for Ellman's reagent preparation.

Stability at +4°C: 1 month.

Ellman's Reagent

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

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

Assay procedure

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

Plate preparation

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

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

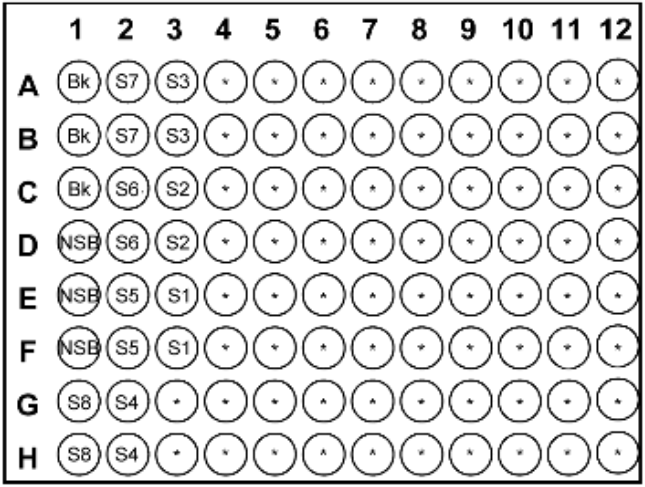
Stability at +4°C: 1 month.

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

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

Plate set-up

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



Bk : Blank S1-S8 : Standards 1-8
NSB : Non Specific Binding * : Samples or Quality Controls

Pipetting the reagents

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

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

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

> ***EIA Buffer***

Dispense 50 µL to Non Specific Binding wells (NSB) wells.

> ***Insulin (human) 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.

> ***Insulin (human) Quality Control and Sample***

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

> ***Insulin (human) Tracer***

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

Incubating the plate

Cover the plate with cover sheet and incubate 1 hour at room temperature.

Developing and reading the plate

- Empty the plate by inverting it. Rinse each well by adding 300 µL of Wash Buffer and repeat washing step 5 times. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

- Add 200µL of Ellman's reagent.

- Cover the plate with cover sheet and incubate in the dark at room temperature for 30 minutes on an orbital microplate shaker at 300 rpm.

- Read the plate at 405 nm or at 414 nm (yellow color) using spectrophotometer plate reader.

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)				
	Blank	NSB	Standard	Sample or QC
EIA Buffer	-	50	-	-
Standard	-	-	50	-
Sample or QC	-	-	-	50
Tracer	-	50	50	50
Cover plate, incubate **60** minutes at room temperature				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
Ellman's reagent	200			
Cover plate, incubate **30** minutes at room temperature while shaking the plate using at 300 rpm on an orbital microplate				
Read the plate at 405 nm or at 414 nm				

Data analysis

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

- Calculate the average absorbance for each NSB, standards, QC and samples.
- For each standard, plot the absorbance (y axis) versus the concentration (x axis) graph. Draw a best-fit line through the points.
- To determine the concentration of samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of unknown samples.
- Samples with a concentration of at least 52.0 µUI/mL must be re-assayed after dilution in EIA Buffer.
- Most plate readers come with a curve-fitting software pre-installed that is capable of generating graphs. It is highly recommended to use this software if available on the device.
  For Insulin ELISA kit the best curve-fitting is obtained with 4-parameter logistic fit (4PL) or 4-parameter logistic fit with 1/Y^2 ponderation. Refer to it for further information.

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

Acceptable range

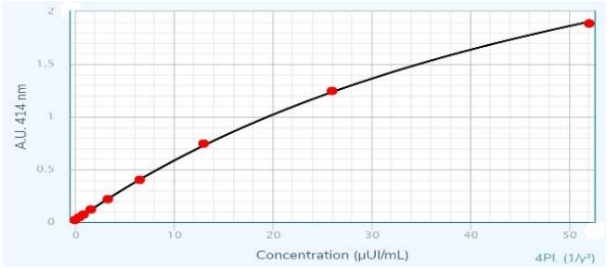
- NSB absorbance ≤ 0.06 A.U.
- Limit of detection ≤ 0.3 µUI/mL
- QC ±25% of the expected concentration (see the label of the QC vial)

Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.
The data was obtained using all reagents as supplied in this kit under the following conditions: 30 minutes developing at room temperature, reading at 414 nm. A 4 parameter logistic fitting with a 1/Y^2 ponderation was used to determine the concentrations.

Standard	Insulin µUI/mL	Absorbance A.U.
S1	52.0	1.885
S2	26.0	1.245
S3	13.0	0.745
S4	6.5	0.402
S5	3.3	0.218
S6	1.6	0.121
S7	0.8	0.072
S8	0.4	0.046
NSB	0.0	0.021

Typical Insulin standard curve



Troubleshooting

Absorbance values are too low:

- one of the reagents was not properly dispensed,
- incorrect preparation,
- assay performed before reagents reached room temperature,
- reading time not long enough.

High signal and background in all wells:

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

High dispersion of duplicates:

- poor pipetting
- irregular plate washing.

These are a few examples of troubleshooting that may occur. If further information or explanation is needed, please contact Bertin Bioreagent Technical Support by phone on +33 (0)139 306 036, fax +33 (0)139 306 299 or by E-mail tech@bertin-bioreagent.com. Please have batch number of the kit (see outside the box) ready to provide to the technical support.

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

Bibliography

1. Grassi J. & Pradelles Ph.
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

2. J. Grassi and P. Pradelles
The use of Acetylcholinesterase as a Universal marker in Enzyme-Immunoassays
Proceedings of the Third International Meeting on Cholinesterases, American Chemical Society (1991)

3. Philippe Pradelles, Jacques Grassi, and Jacques Maclouf
Enzyme Immunoassays of Eicosanoids Using Acetylcholinesterase
Methods in enzymology, vol. 187, p24, 1990

4. Gisela Wilcox
Insulin and Insulin Resistance
Clin Biochem Rev, vol 26, p19, May 2005

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

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

To offer a complete solution to researchers, Bertin Health & Life Sciences offers a range of unique laboratory equipment from Air Sample collection and Sample Homogenisation.

Our products are available worldwide through us directly or via our distributor network. Our sales team is active on all continents and will be delighted to answer all your commercial questions.

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CONTACT US

Bertin Technologies

10 bis Avenue Ampère

Parc d'Activités du Pas du Lac 78180

Montigny-le-Bretonneux FRANCE

+33 (0)139 306 036

tech@bertin-bioreagent.com

EU webstore: Bertin-bioreagent.com
US webstore: Bertin-corp.com