bertin bioreagent

ELISA

TRAINING KIT

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

ELISA TRAINING KIT

#B05005.2x96 wells

#B05006.96 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

#B11005
Version: 0124

Table of contents

96 wells
Storage: +4°C
Expiry date: stated on the package

The kit #B05005 has been designed to be used by 24 students and to perform 4 samples in duplicate, it contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
96-well Microtiter plate, pre-coated with mouse anti β-lactoglobulin	Blister with zip	B08003.1 ea	2	-
β-lactoglobulin (BLG) Tracer	Green	B04003.100 dtn	2	Lyophilised
β-lactoglobulin (BLG) Standard	Silver	B06003.1 ea	1	Lyophilised
Ellman's Reagent 50	Black with red septum	A09000_50.100 dtn	3	Lyophilised
Technical Booklet		B11005	1	-

The kit is also available in half-size format under the reference #B05006:

Designation	Colour of cap	Item #	Quantity per kit	Form
96-well Microtiter plate, pre-coated with mouse anti β-lactoglobulin	Blister with zip	B08003.1 ea	1	-
β-lactoglobulin (BLG) Tracer	Green	B04003.100 dtn	1	Lyophilised
β-lactoglobulin (BLG) Standard	Silver	B06003.1 ea	1	Lyophilised
Ellman's Reagent 50	Black with red septum	A09000_50.100 dtn	2	Lyophilised
Technical Booklet		B11005	1	-

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.

β-lactoglobulin (BLG)

β-lactoglobulin (BLG) is a relatively small protein of 162 amino-acid residues with a 183 kDa molecular weight. β-lactoglobulin is the major protein of cow and ewe milk (~3 g/L), just after casein. It is also present in many other mammalian species, a notable exception being humans.
Its structure, properties and biological role have been extensively reviewed.

β-lactoglobulin is a major molecule of interest in food industry since its characteristics can be either an advantage or a drawback in dairy products and for their processing. Absent from human milk, it is
suspected to cause allergies to human.

The training ELISA kits specifically measure the bovine β-lactoglobulin ***[4, 5]***.

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 β-lactoglobulin (BLG).

β-lactoglobulin (BLG) introduced into the wells (standard or sample) is bound by the monoclonal antibody coated on the plate. An acetylcholinesterase (AChE) - Fab' conjugate which binds selectively to a different epitope on the beta-lactoglobulin molecule, is also added to the wells.

The two antibodies then form a sandwich by binding on different epitopes of the β-lactoglobulin (BLG).

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

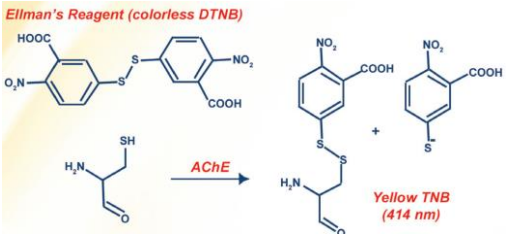
The concentration of β-lactoglobulin (BLG) is determined by measuring the enzymatic activity of immobilized Tracer using Ellman's reagent. AChE tracer acts on Ellman's Reagent to form a yellow compound that strongly absorbs at 405 nm or at 414 nm.

The intensity of colour, which is determined by spectrophotometry, is proportional to the amount of β-lactoglobulin (BLG) present in the well during the immunological reaction.

Comparing the absorbance of one solution of known concentration (called standard) with the absorbance of your samples, you may determine the concentration of your

sample. For more accurate results, you may also use several standard solutions having different known concentrations. By plotting the absorbance of each standard on a graph, you may draw up a standard curve.

Samples of unknown concentration will develop a yellow color corresponding to an absorbance value. Reporting this absorbance on the standard curve will allow you to determine the concentration.



Operating procedure

This assay follows a 5-step procedure:

Dispensing the samples and controls

Dispense each positive/negative control and sample (water, cow milk, biscuit juice,…) in a well, then add Tracer (AChE labelled antibody).

Standard BLG will serve as positive control. Water as

negative control will allow the detection of non-specific signal due to unspecific binding of antibodies on the wells.

Immunological reaction

Wait for immunologic reaction. This reaction will take place in about 30 minutes at room temperature.

Washing the plate

Wash the plate to get rid off of unbound antibodies.

Revelation

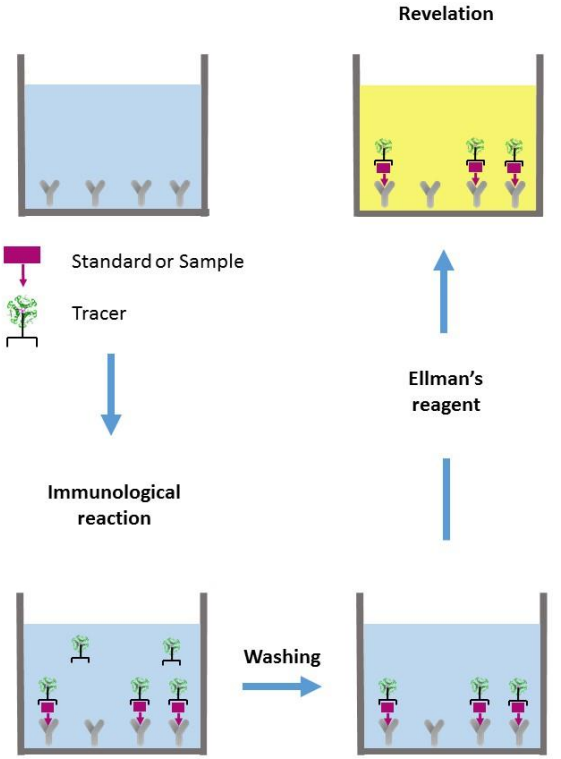
Add in each well Ellman's Reagent (AChE substrate).

Allow the enzymatic reaction to perform. This leads to a yellow colour apparition proportional to the amount of AChE immobilised on the plate (Tracer).

Reading the plate

After 15 minutes, the yellow colour might be read on a spectrophotometer at 405-414 nm or visualized directly.

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 (10 to 1000 µL)
- Spectrophotometer plate reader (405 nm or 414 nm filter)
- Disposable tips
- Samples to be tested

Sample preparation

This kit recognises specifically bovine β-lactoglobulin.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after preparation.
- We advise to use mineral water for the assay to avoid organic contamination that would inhibit AChE.
- Do not use mineral water that contains a large amount of divalent cations (Ca^{2+} or Mg^{2+}) that will precipitate the phosphate buffer present in the reagents.

Sample preparation

Dilute one drop of cow milk in 1 liter of water.

Many other food samples (liquid or solid) containing traces of cow milk may be used, providing that the correct dilution has been checked. Indeed, a large excess of β-lactoglobulin may bind all the antibodies (tracer and coating) leading to very low signal (hook effect). Without precise indication, serials dilutions shall be tested to find the optimum dilution. For solid samples (cookies, cakes, cheese, ...), homogenize in water.

Do not store the diluted samples.

The presence of β-lactoglobulin might also be tested in hypoallergenic milks. To lower the risk of allergy, those milks are treated with proteases that cut the β-lactoglobulin which cannot be recognised anymore by the antibodies.

Reagent preparation

Reagents are provided lyophilised. While opening, be careful not to discard powder.

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

β-lactoglobulin (BLG) Tracer

Reconstitute each Tracer vial # B04003 with 10 mL of water. Allow it to stand for 5 minutes or until it is completely dissolved. Mix tracer thoroughly by gentle

inversions.

Stability at +4°C: 4 weeks.

β-lactoglobulin (BLG) Standard

Reconstitute the Standard vial #B06003 with 5 mL of water. Allow it to stand for 5 minutes or until it is completely dissolved. Mix standard thoroughly by gentle inversions.

This solution (100ng/mL) will be used as positive Control.

Stability at 4°C: 4 weeks

Ellman's Reagent

5 minutes before use (development of the plate), reconstitute one vial of Ellman's Reagent # A09000_50 with 50 mL of water. The tube content should be thoroughly mixed.
Only 20 mL are necessary for one plate.
We provide 2 or 3 vials to enable 2 or 3 sets of different experiments.

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

Practical handling

Distribution of reagents

We suggest the following:

- Each group will handle one 8-well strip
- Each dispatch will be done in duplicate

Plate preparation

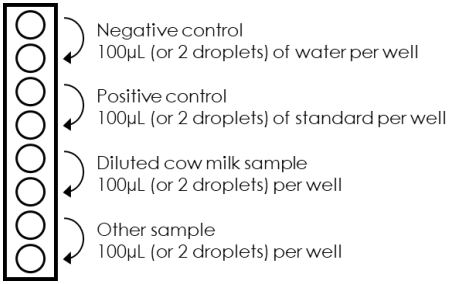
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.

Samples and Controls dispensing

Dispatch 100 µL (or 2 droplets) of Control or sample (solution to be tested) as indicated below.

Tracer

Dispense 100 µL (or 2 droplets) of Tracer in each well, starting from negative Control.



Incubating the plate

- Allow the plate to stand at room temperature (immunological reaction) for 30 minutes. This step can be accelerated by increasing the temperature (i.e. 15 to 20 minutes at 30°C). Be careful not to exceed +37°C which would inactivate the AChE.

- Empty the plate by turning it over. Wash the wells thoroughly with water (phosphate buffer can also be used). Empty the plate and blot it on a paper towel to discard any trace of liquid.

Developing and reading the plate

- Dispense 200 µL (4 droplets) of Ellman's Reagent to each well. Optimum development is obtained at room temperature.
- In order to avoid the direct light exposure, it is recommended to cover the plate with aluminium foil.
- The absorbance is read at a wavelength between 405 and 414 nm on a spectrophotometer 15 minutes after the addition of Ellman's Reagent. If you don't have a spectrophotometer device, you can monitor the colour development by direct visualization.
  It is possible to rinse the plate and perform a second revelation, without affecting the performance of the assay.

Data analysis

Compare the colour formation in the wells containing the positive control to the other samples. If you have performed a spectrophotometer reading, β-lactoblogulin concentration can be calculated using the following formula:

$$[BLG] = \frac{\text{Sample (A.U.)}}{\text{Standard (A.U.)}} \times 100 \times F$$

Where:

[BLG]:	β-lactoglobulin concentration expressed in ng/mL
Sample (A.U.):	Sample in absorbance units
Standard (A.U.):	Standard in absorbance units
100:	β-lactoglobulin concentration of Standard solution (100 ng/mL)
F:	Sample dilution factor

In the case of cow milk, dilution factor is 20 000 (one droplet of approximately 50µL in 1 litre of water)

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 according to the protocol and 15 minutes incubating time.

Negative Control	0.009 and 0.010 A.U.
Positive Control	0.769 and 0.796 A.U.
Diluted cow milk	0.574 and 0.571 A.U

In this case the β-lactoglobulin concentration is 1.46 g/L or 1.46×10^6 ng/mL.

Training questions

- Give an interpretation of what happens in each well with a schematic drawing.
- Justify how the colour intensity is related to β-lactoglobulin concentration.
- Knowing the general structure and properties of proteins, could you provide a general conclusion?

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.

> ***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.

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.

Bibliography

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Enzyme Immunoassays of Eicosanoids Using Acetylcholinesterase
Methods in enzymology, vol. 187, p24, 1990

4. Negroni L, Bernard H, Clement G, Chatel J.M, Brune P, Frobert Y, Wal J.M, Grassi J,
Two-site enzyme immunometric assays for determination of native and denatured β-lactoglobulin
Journal of Immunological Methods 200 (1998), 25-37

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Production and Epitopic Characterization of Monoclonal Antibodies Against Bovine β-Lactoglobulin.
Dairy Sci (1997), 80:1977-1987

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.

Should you need help with a product, you can contact our technical support by emailing to tech@bertin-bioreagent.com
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