PROLACTIN (MOUSE)
ENZYME IMMUNOASSAY KIT
96 Wells

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

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PROLACTIN (MOUSE) EIA KIT (A05136)

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

This kit contains:

- A covered 96 wells plate, pre-coated with mouse anti-rabbit IgG (#A08100)
- 1 vial of mouse prolactin tracer (#A04136)
- 1 vial of mouse prolactin antiserum (#A03136)
- 2 vials of mouse prolactin standard (#A06136)
- 2 vials of mouse prolactin quality control (QC) (#A10136)
- 1 vial of mouse prolactin EIA buffer, lyophilized (#A07136)
- 1 vial of concentrated wash buffer, liquid (#A17000)
- 1 vial of tween 20, liquid (#A12000)
- 2 vials of Ellman's reagent, lyophilized (#A09000)
- 1 instruction booklet (#A11136)
- 1 template sheet
- 1 well cover sheet

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.

PRECAUTIONS FOR USE

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

Each time a new pipet tip is being used, aspirate a sample or reagent and dispense it back into the same vessel. Repeat this operation two or three times before distribution.

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

Wearing gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

GENERAL COMMENTS

Prolactin is a polypeptide made of 197 (22560 Da) amino-acids residues [1].

The three dimensional structure is tetra helical [2].

The pituitary gland is the main source of prolactin but other organs such as immune cells, brain, reproductive organ, help to maintain the physiological prolactin level.

This polypeptide is defined as an hormone-cytokine with endocrine, paracrin and autocrine functions[3].

More than 300 distinct biological functions of prolactin have been recorded, due to the fact that the receptor is ubiquitous.

It could be possible to divide the prolactin effect into 4 different areas: reproduction, immune function, osmoregulation, metabolism and tumorogenesis [4] .

PRINCIPLE OF THE ASSAY

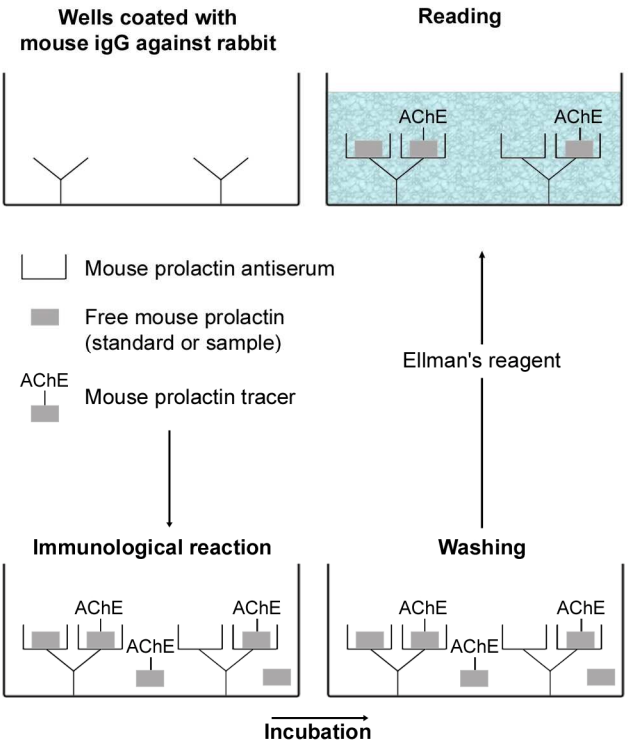
This Enzyme Immunoassay (EIA) is based on the competition between unlabelled mouse prolactin and acetylcholinesterase (AChE) linked to mouse prolactin (tracer) for limited specific rabbit anti-mouse prolactin antiserum sites.

The complex rabbit antiserum-mouse prolactin (free prolactin or tracer) binds to the mouse monoclonal anti-rabbit antibody that is attached to the well.

The plate is washed to eliminate the unbound tracer 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 is determined by spectrophotometry and is inversely proportional to the amount of free mouse prolactin present in the well during the immunological incubation.

The principle of the assay is summarised on the following page


Wells coated with
mouse igG against rabbit
Reading
AChE
AChE
Mouse prolactin antiserum
Free mouse prolactin
(standard or sample)
AChE
Mouse prolactin tracer
Ellman's reagent
Immunological reaction
Washing
AChE
AChE
AChE
AChE
AChE
AChE
Incubation

MATERIALS AND EQUIPMENT REQUIRED

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

FOR THE ASSAY

- ☞ Precision micropipettes (10 to 1000 µL)
- ☞ Spectrophotometer plate reader (405 or 414 nm filter)
- ☞ Microplate orbital shaker
- ☞ Ultra pure water (#A07001)
- ☞ Polypropylene tubes

SAMPLE COLLECTION & PREPARATION

This assay may be used to measure mouse prolactin in culture media. Other media not validated.

GENERAL PRECAUTIONS

☞ All samples must be free of organic solvents prior to the assay.

☞ Samples should be assayed immediately after collection or should be stored at –20°C

REAGENT PREPARATION

☞ EIA buffer
Reconstitute one vial with 50 mL of ultra pure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 1 month.

☞ Mouse prolactin standard
Reconstitute the standard with 1 mL of culture media used in the experiment. Stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
The concentration of the first standard is 250 ng/mL. Prepare seven propylene tubes (for the seven other standards) and add 500 µL of culture media in each tube. Add 500 µL of the first tube (containing the first standard) to the second tube. Continue this procedure for the other tubes. Thus, standard concentrations are: 250 (S1), 125 (S2), 62.5 (S3), 31.3 (S4), 15.6 (S5), 7.81 (S6), 3.91 (S7) and 1.95 (S8) respectively.
Stability at +4°C: 1 day

☞ Quality Control (QC)
Reconstitute the vial with 1 mL of culture media used in the experiment. Stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 1 day.

☞ Mouse prolactin-AChE tracer
Reconstitute the vial with 5 mL of mouse prolactin EIA buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability at +4°C: 1 month.

☞ Mouse prolactin antiserum
Reconstitute the vial with 5 mL of mouse prolactin 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 1 mL of the concentrated wash buffer with 400 mL of ultra pure water. Add 200 µL of tween 20 (use a magnetic stirrer to mix the contents).
Stability at +4°C: 1 week.

☞ Ellman's reagent
Five minutes before use, reconstitute with 50 mL of distilled or deionized water. The tube contents 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

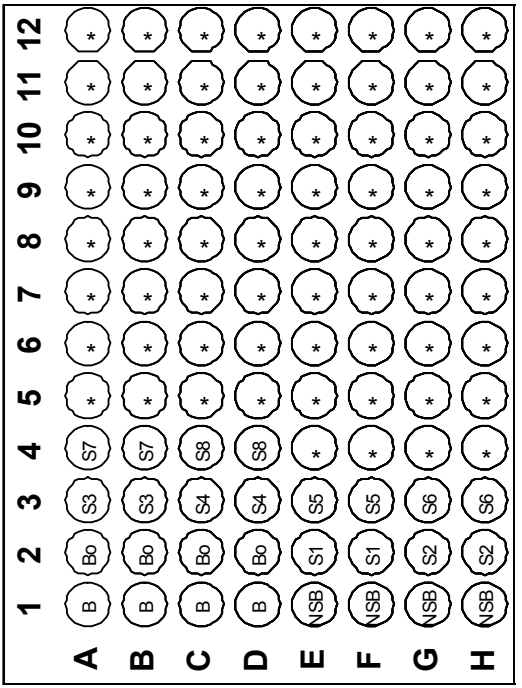
Prepare the wash buffer as indicated in the reagent preparation section. Open the plate packet and select sufficient strips for your assay. The remaining strips must be replaced in the packet and stored at -20°C. Rinse each well five times with the wash buffer (300 µL/well).
Just before distributing reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on the following page. The contents of each well may be recorded on the sheet provided with the kit.

PIPETTING THE REAGENTS

Note that the first column must dedicated for the blank (B) and the Non-Specific Binding (NSB).
All samples and reagents must reach room temperature prior to performing the assay



B: Blank
NSB: Non-Specific Binding
Bo: Maximum Binding
S1-S8 Standards 1-8
*: Samples - Qc

Use different tips to pipet the buffer, standard, sample, tracer, antiserum and other reagents.

- EIA buffer
  Dispense 100 µL to Non-Specific Binding (NSB) wells and 50 µL to Maximum Binding (Bo) wells.

- Mouse prolactin standard
  Dispense 50 µL of each of the eight standards (S1 to S8) in duplicate into the appropriate wells. Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

- Samples/Quality control
  Dispense 50 µL in duplicate to appropriate wells.

- Mouse prolactin AChE tracer
  Dispense 50 µL to each well, except the Blank (B) wells.

- Mouse prolactin antiserum
  Dispense 50 µL to each well except the Blank (B) wells and Non Specific Binding (NSB) wells.

INCUBATING THE PLATES

Cover the plate with a plastic film and incubate for 16-20 hours at room temperature (an optimal temperature of +20°C is suggested)

ASSAY SUMMARY

Enzyme Immunoassay Protocol (Volume in µL)					
	Blank	NSB	Bo	Standard	Sample
EIA buffer	150	100	50	-	
Standard	-			50	-
Sample	-				50
Tracer	-	50			
Antiserum	-	50			
Cover the pate and incubate at +20°C for 16-20 hours					
Wash the plate 5 times					
Ellman's reagent	200				
incubate the plate with orbital shaker in the dark at +20°C					
Read the plate between 405 and 414 nm					

DEVELOPING AND READING THE PLATE

Reconstitute the wash buffer and Ellman's reagent as indicated in the reagent preparation section. Empty the plate by turning it over and shaking it. Then, wash each well five (5) times with the wash buffer (300 µL/well).

Dispense 200 µL of Ellman's reagent to the 96 wells. Incubate in the dark (plate covered with an aluminium sheet) at room temperature. Optimal development is obtained using an orbital shaker.

The plate should be read between 405 and 414 nm (yellow colour) when the Maximum Binding (Bo) wells reach an absorbance of 200 mAU after blank subtraction

DATA ANALYSIS

Make sure that your plate reader has subtracted the absorbance readings of the blank well (absorbance of Ellman's reagent) from the absorbance readings of the rest of the plate. If not, do it now.

- Calculate the average absorbance for each NSB, Bo, standards and samples.
- Calculate the B/Bo (%) for each standard and sample: (average absorbance of standards or sample - average absorbance of NSB) divided by (average absorbance of Bo - average absorbance of NSB) & multiplied by 100.
- Using a semi-log graph paper, plot the B/Bo (%) 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, find the B/Bo (%) value 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 250 ng/mL should be re-assayed after dilution in culture media.

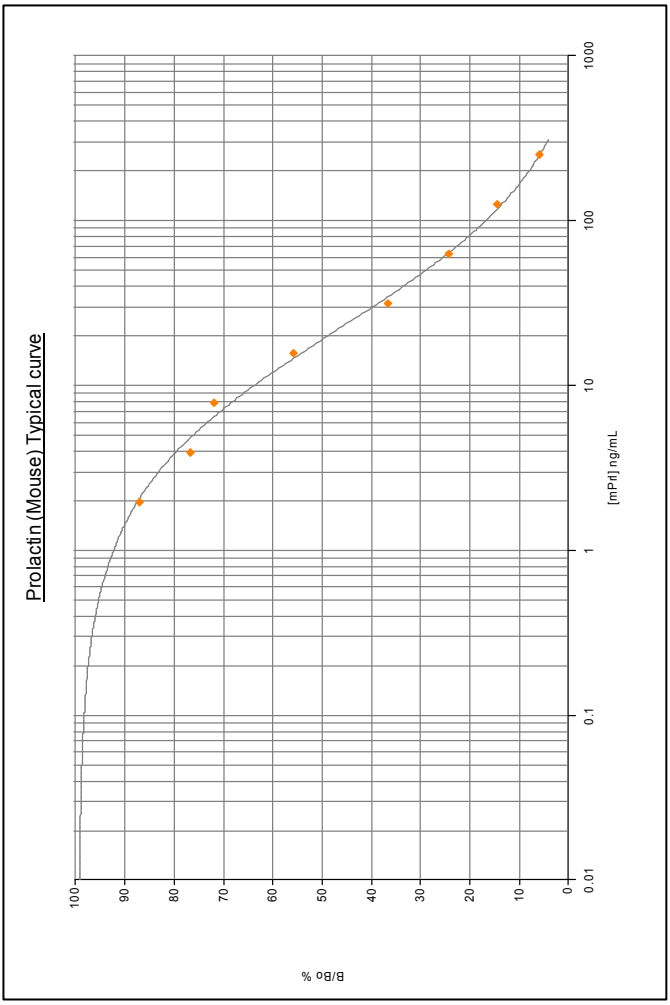
Most plate readers are supplied with curve-fitting software capable of graphing this type of data (autospline, logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information

TYPICAL DATA

EXAMPLE DATA

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 four-parameter logistic curve fitting was used to determine the concentrations.

	mAU	B/Bo (%)
NSB	N/A	N/A
Bo	211	100
Standard 250 ng/mL	12	6
Standard 125 ng/mL	28	13
Standard 62.5 ng/mL	47	22
Standard 31.2 ng/mL	72	34
Standard 15.6 ng/mL	109	52
Standard 7.81 ng/mL	140	67
Standard 3.9 ng/mL	150	71
Standard 1.95 ng/mL	170	81


Prolactin (Mouse) Typical curve
B/Bo %
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
[mPrl] ng/mL

ACCEPTABLE RANGE

- ☞ Ratio NSb absorbance / Bo absorbance: < 0.1
- ☞ Bo absorbance: >200 mAU after blank subtraction in the conditions indicated above.
- ☞ 50% B/Bo%: 25 ng/mL

ASSAY VALIDATION AND CHARACTERISTICS

The enzyme immunoassay of mouse prolactine has been validated to be used in cell culture media.

☞ Limit of detection : 1.7 ng/mL
The limit of detection is a calculated value, it is the concentration of mouse prolactine obtained from the Bo minus 3 standard deviations.

☞ Intra-assay variation

QC level	Concentration (ng/mL)	CV (%)
QC High	94	12%
QC Medium	59	6%
QC Low	4	16%

☞ inter-assay variations

QC level	Concentration (ng/mL)	CV (%)
QC High	97	13%
QC Medium	62	10%
QC Low	5	22%

☞ Cross reactivity

Compound	Cross-reactivity
TSH (mouse)	< 1%
LH (mouse)	< 1%
GH (mouse)	< 1%
Prolactin (rat)	1.4 %

ASSAY TROUBLE SHOOTING

- ☞ Bo value is too low: incubation has been done in wrong conditions (time or temperature) or reading time is too short or mouse prolactin-AChE tracer, mouse prolactin antiserum or Ellman's reagent have not been dispensed.
- ☞ NSB value is too high: contamination of NSB wells with mouse prolactin antiserum or inefficient washing.
- ☞ High dispersion of duplicates: poor pipetting technique or irregular plate washing.
- ☞ IC_{50} not within the expected range: wrong preparation of standards.

These are a few examples of trouble shootings that may occur. If you need further explanation, Bertin Pharma Pharma will be happy to answer any questions or information about this assay.

Please feel free to contact our technical support staff by letter, phone: 33 (0)1 39 30 60 36, fax: 33 (0)1 39 30 62 99 or E-mail: bioreagent@bertinpharma.com, and be sure to indicate the lot number of the kit (see outside the box).

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