bertin bioreagent

Example Version

HISTAMINE

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

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

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

Example Version

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

Histamine

ELISA kit

#A05890.96 wells

Example Version

For research laboratory use only
Not for human diagnostic use

This assay was developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11890

Version: 0124

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Example Version

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

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
Histamine precoated 96-well Strip Plate	Blister with zip	A08890.1 ea	1	-
Histamine Tracer	Green	A04890.100 dtn	1	Lyophilised
Histamine Standard	Blue screw cap	A06890.1 ea	2	Liquid
Derivatization Reagent	White with red septum	A15890.1 ea	2	Powder
Derivatization Buffer	Silver/Pink	A16890.1 ea	1	Liquid
Histamine ELISA Buffer	Silver/Blue	A07890.1 ea	1	Lyophilised
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween 20	Transparent	A12000.1 ea	1	Liquid
Histamine Quality Control	Green screw cap	A10890.1 ea	2	Liquid
Ellman's reagent 50	Black with red septum	A09000_50.100 dtn	2	Lyophilised
Technical booklet	-	A11890	1	-
Well cover sheet	-	-	1	-

Example Version

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 100 µg 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), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Example version

Background

Acetylcholinesterase AChE® Technology

Acetylcholinesterase (AChE®), the enzymatic label for ELISA, 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 ELISA is patented by the French academic research Institute CEA ***[1, 2, 3]***, and Bertin Bioreagent has expertise to develop and produce 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 ELISAs:

- **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 labelling 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.

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- **Wide dynamic range:** AChE® is a stable enzyme and its activity remains constant for many hours, 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), 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.

Histamine

Histamine is a natural amine present in many animal and human tissues. It was considered to be the mediator of immediate allergy.

Histamine plays a role in various physiological processes, such as control of gastric acid secretion, neurotransmission and modulation of inflammatory and immunological reactions.

Example version

Principle of the assay

The enzymatic immunoassay (ELISA) is based on the competition between unlabelled (free) Histamine (standards / QC / samples) and acetylcholinesterase (AChE) linked to Histamine (Tracer) for limited specific mouse anti-Histamine antibody sites.

As a former step of this assay, Histamine is derivatized to increase the affinity of Histamine to the antibody and consequently increase the sensitivity of the assay.

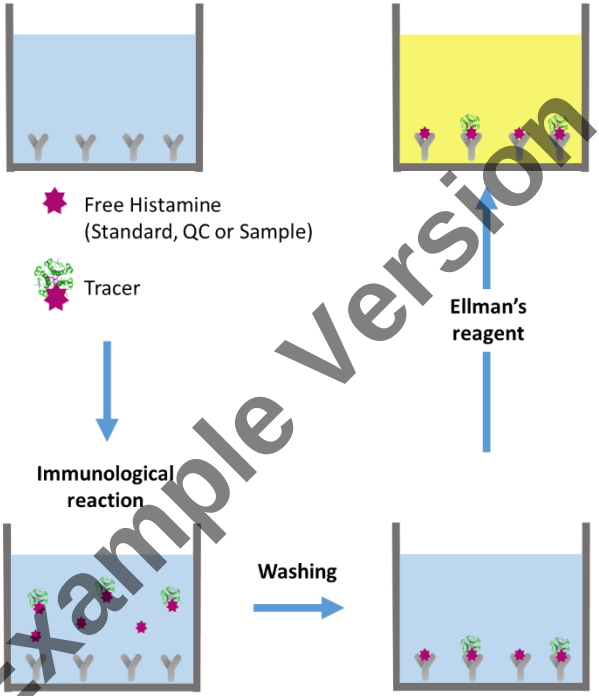
Tracer and free Histamine are incubated in wells which have been precoated with a mouse anti-Histamine antibody. The plate is washed to remove any unbound reagent, then Ellman's Reagent (enzymatic substrate for AChE and chromogen) is added to the wells.

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 Histamine present in the well during the immunological incubation.

Example Version

The principle of the assay is summarised below:



Example Version

Assay characteristics

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography ***[4, 5]***.

- ***Limit of detection (LOD):*** 0.5 nM (calculated as the concentration of Histamine corresponding to the B0 average minus three standard deviations)

- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
Histamine	100 %
Histidine	< 0.01 %
1-methylHistamine	0.01 %
3-methylHistamine	0.038 %
Serotonin	< 0.01 %

- ***Intra & Inter-assay variation***

	Plasma QC (2.2 nM)	Plasma QC (8.6 nM)	Plasma QC (33.6 nM)
Mean value	-	7.8	32.6
Number of values	30	30	30
Intra-assay coeff. of variation (%)	13.6	7.4	15.1

Example Version

Inter-assay coeff. of variation (%)	19.4	8.0	15.8
Recovery (%)	-	90.7	97.1
Confidence Interval	-	90.71 ± 2.74	97.08 ± 5.78

	Buffer QC (1 nM)	**Buffer QC (5 nM)**	**Buffer QC (30 nM)**
Mean value	1.2	5.2	32.8
Number of values	30	30	30
Intra-assay coeff. of variation (%)	16.3	9.8	21.4
Intra-assay coeff. of variation (%)	28.9	11.6	21.4
Recovery (%)	123.2	103.4	109.5
Confidence Interval	123.15 ± 13.39	103.41 ± 4.51	109.47 ± 8.80

	Cell culture medium QC (5 nM)	**$HClO_4$ QC (5 nM)**
Mean value	4.58	5.47
Number of values	20	20
Intra-assay coeff. of variation (%)	7.07 %	6.43 %
Inter-assay coeff. of variation (%)	5.20 %	3.97 %
Recovery (%)	91.60 %	109.40 %

Example Version

> *Dilution test*

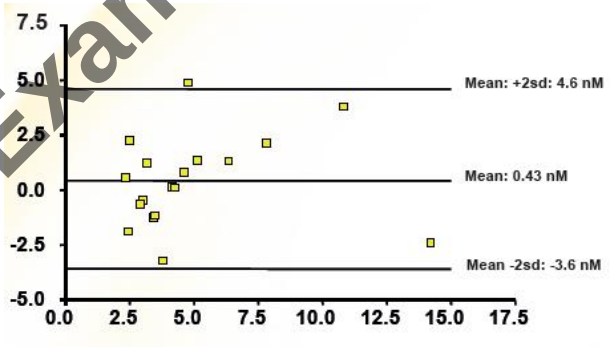
Day	Dilution	Histamine measured	Corrected concentrations	Recovery (%)	Mean
1	1/1	30.79	30.79	94.39	136.41
	1/5	9.40	47.00	144.08	
	1/10	5.08	50.80	155.73	
	1/20	2.47	49.40	151.44	
2	1/1	32.15	32.15	98.56	113.65
	1/5	8.59	43.00	131.82	
	1/10	3.45	34.56	105.95	
	1/20	1.92	38.58	118.27	
3	1/1	31.94	31.94	97.92	130.08
	1/5	8.77	43.83	134.37	
	1/10	4.43	44.31	135.84	
	1/20	2.48	49.65	152.21	
4	1/1	26.56	26.56	81.42	115.24
	1/5	8.17	40.85	125.23	
	1/10	3.86	38.58	118.27	
	1/20	2.22	44.38	136.05	
5	1/1	22.15	22.15	67.90	107.72
	1/5	7.10	35.50	108.83	
	1/10	3.75	37.50	114.96	
	1/20	2.27	45.40	139.18	

Example Version

> *Recovery test*

	Histamine added (nM)	Histamine measured (nM)	Recovery (%)
Histamine ELISA Buffer	5	5.2	103
Cell culture medium	5	5.04	101
Cell culture medium & additives	5	4.58	91.6
$HClO_4$	5	5.47	109

> *Comparison with a reference method on 18 samples*



Example Version

> *Stability test (freezing / thawing)*

	Plasma QC1		Plasma QC2	
	32.6 nM	**Recovery (%)**	**7.8 nM**	**Recovery (%)**
1 cycle	26.69	81.9	8.49	108.9
2 cycles	29.42	90.3	9.63	123.5
3 cycles	30.76	94.4	8.22	105.4
4 cycles	24.26	74.4	6.73	86.3
5 cycles	-	-	7.78	99.7
Mean	27.78	-	8.17	-
Standard deviation	2.90	-	1.06	-
CV (%)	10.42	-	12.93	-

	Buffer QC1		Buffer QC2		Buffer QC3	
	30 nM	**Recovery (%)**	**5 nM**	**Recovery (%)**	**1 nM**	**Recovery (%)**
1 cycle	36.67	122.2	6.88	137.6	1.36	136.0
2 cycles	29.63	98.8	4.17	83.4	1.29	129.0
3 cycles	34.30	114.3	5.62	112.4	1.83	183.0
4 cycles	29.83	99.4	5.69	113.8	1.31	131.0
5 cycles	30.17	100.6	6.94	138.8	1.84	184.0
Mean	32.12	-	5.86	-	1.53	-
Standard deviation	3.19	-	1.13	-	0.28	-
CV (%)	9.93	-	19.6	-	18.56	-

Example Version

Materials and equipment required

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

- Anhydrous N,N-Dimethylformamide (DMF)(*) (if not available, DMS0)
- Precision micropipettes (20 to 1000 µL)
- Multichannel pipette and disposable tips 30-300µL
- Spectrophotometer plate reader (405 or 414 nm filter)
- Microplate washer (or wash bottles)
- UltraPure water #A07001.1L
- Orbital microplate shaker
- Polypropylene tubes (**no glass tubes**)
- For plasma sample: EDTA tubes
- For solid and liquid biological samples: $HClO_4$ & NaOH

(*) Make sure that the bottle of DMF has been opened for a short period of time. This point is important to get a good derivatization rate.

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Otherwise, organic contamination can significantly affect the enzymatic activity of the tracer Acetylcholinesterase (AChE).
Do not use distilled water, HPLC-grade water or sterile water.

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

Example Version

Sample collection and 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.

This assay may be used to measure Histamine in samples such as plasma, urine, culture supernatants as well as liquid (e.g. broncho-alveolar lavage fluids) or solid (brain, nervous tissues) biological samples after extraction. Please refer to the appropriate paragraph for your sample preparation protocol.

Plasma

Collect blood samples in tubes containing EDTA. Centrifuge the samples at 1,600 g for 20 minutes. Collect plasma and keep at -20°C until assay. Thaw the sample on the day of the assay, vortex and centrifuge it at 1,600 g for 20 minutes to eliminate the fibrin.

No prior extraction procedure is necessary to measure Histamine in plasma samples. If necessary, plasma samples may be diluted in Histamine EIA buffer before derivatization (see below).

Example Version

Urine or culture supernatants

Collect samples in polypropylene tubes. Store the samples at -20°C until assay. No prior extraction is necessary to measure Histamine in such samples.

Liquid or solid biological samples

- For solid samples, we recommend addition of $HClO_4$ which will precipitate large proteins, while Histamine will remain in solution. Samples must be extracted at room temperature with 0.1M Perchloric Acid final concentration (10 µL/mg of tissue).

  Homogenize and then centrifuge at 10,000 g for 5 minutes. Collect the supernatant.

- Liquid samples can be stored at -20°C just after collection in polypropylene vials. Before assaying, you have to filter liquid samples through a 0.22 µm filter in a tube containing $HClO_4$ 1M to get a final $HClO_4$ concentration of 0.1 M (for example: your tube must contain 20 µL $HClO_4$ 1M for 200 µL of sample).

  Homogenize and then centrifuge at 10,000 g for 5 minutes. Collect the supernatant.

Example version

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.

If you want to use the kit in two times, we provide one additional vial of Standard, one of Derivatization Reagent, one of Quality Control and one of Ellman's Reagent.

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

Histamine ELISA Buffer

Reconstitute the Histamine ELISA Buffer #A07890 with 25 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 week

Histamine Standard

The Assay Buffer used to prepare the standards depends on the sample to be assayed:

- for plasma or urine samples, prepare the Standards using the Histamine ELISA Buffer,
- for culture supernatant samples, prepare the Standards using the same culture medium as for the sample,
- for extracted liquid or solid samples, prepare the Standards in 0.1 M $HClO_4$.

Example Version

Prepare one Histamine Standard vial #A06890 with 900 µL of Assay Buffer. Mix carefully 3 times with the pipet tip.

The obtained solution, called S0, has a concentration of 500 nM.

Take 8 polypropylene tubes (for the eight standards S1 to S8) and prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of Assay Buffer	Standard concentration
S1	100 µL of S0	900 µL	50 nM
S2	500 µL of S1	500 µL	25 nM
S3	500 µL of S2	500 µL	12.5 nM
S4	500 µL of S3	500 µL	6.25 nM
S5	500 µL of S4	500 µL	3.13 nM
S6	500 µL of S5	500 µL	1.56 nM
S7	500 µL of S6	500 µL	0.78 nM
S8	500 µL of S7	500 µL	0.39 nM

Stability at 4°C: 24 hours

Histamine Quality Control

Prepare one histamine Quality Control vial #A10890 with 900 µL of assay medium, as for the standard. Then dilute 100 µL of QC in 900 µL of assay medium. The final concentration of this QC is labelled on the vial.

Stability at 4°C: 24 hours

Example version

▷ Derivatization reagent

Before use, reconstitute the vial #A15890 with 1 mL of N-N-dimethylformamide (DMF). The kit was validated with fresh DMF; if not available, one can use 1 mL DMSO. Vortex the content, and place the vial in a water bath at 37 °C. The resuspended content can contain tiny particles. This will not interfere with the assay. This reagent cannot be stored.

▷ Histamine-AChE Tracer

Reconstitute the vial #A04890 with 10 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

▷ 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 contents.

Stability a +4°C: 1 month

▷ 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 contents should be thoroughly mixed.

Stability at 4°C and in the dark: 24 hours

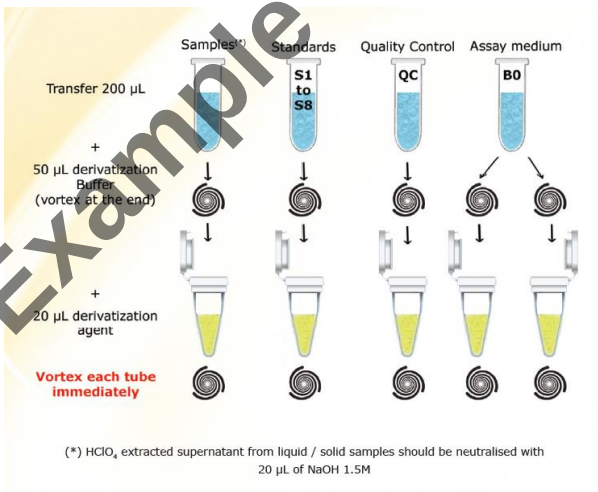
Example version

Assay procedure

It is recommended to perform the assay in duplicate and to follow the instructions hereafter.
Before derivatization, highly concentrated samples may be diluted in Histamine ELISA buffer (plasma, urine) or in assay medium (cell culture medium, $HClO_4$, …)
The assay procedure depends of the sample to be assayed.

Sample derivatization

Illustrated derivatization protocol – please follow carefully the detailed protocol hereafter



Example Version

> ***Histamine ELISA buffer, plasma, urine, culture supernatant:***

- Prepare one polypropylene tube for each standard, quality control and samples. In these polypropylene tubes, distribute:
  - 200 µL of standard, quality control or sample,
  - 50 µL of derivatization buffer
- In two polypropylene tubes that will allow evaluation of maximum binding (B0), distribute:
  - 200 µL of assay medium
  - 50 µL of derivatization buffer
- Vortex all tubes
- **Then tube by tube**:
  - add 20 µL of derivatization reagent
  - **vortex each tube immediately**.

> ***Liquid or solid biological sample:***

- Prepare one polypropylene tube for each standard, quality control and samples. In these polypropylene tubes, distribute:
  - 200 µL of standard, quality control or sample,
  - 20 µL of 1.5M NaOH
  - 50 µL of derivatization buffer
- In two polypropylene tubes that will allow evaluation of maximum binding (B0), distribute:
  - 200 µL of assay medium
  - 20 µL of 1.5M NaOH
  - 50 µL of derivatization buffer
- Vortex all tubes

Example Version

- **Then tube by tube**:
  - add 20 µL of derivatization reagent
  - **vortex each tube immediately**.

▷ Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate pouch and select enough strips for your assay and place the unused strips back in the pouch, store at +4°C for 1 month maximum.

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 blot the last drops by tapping it on paper towels.

▷ Plate set-up

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

Example Version

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bk	S7	S3	*	*	*	*	*	*	*	*	*
B	Bk	S6	S2	*	*	*	*	*	*	*	*	*
C	B0	S6	S2	*	*	*	*	*	*	*	*	*
D	B0	S5	S1	*	*	*	*	*	*	*	*	*
E	B0	S5	S1	*	*	*	*	*	*	*	*	*
F	S8	S4	*	*	*	*	*	*	*	*	*	*
G	S8	S4	*	*	*	*	*	*	*	*	*	QC
H	S7	S3	*	*	*	*	*	*	*	*	*	QC

Bk : Blank
QC : Quality Control
B0 : Maximum Binding
S1-S8 : Standards 1-8
* : Samples

Example version

Pipetting the reagents

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

Use different tips for buffer, standard, sample, tracer 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.

Assay medium

Pool the 2 vials of B0 and dispense 100 µL to Maximum Binding (B0) wells.

> ***Histamine Standard***

Dispense 100 µL of each of the eight derivatized 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.

> ***Histamine Quality Control and Samples***

Dispense 100 µL in duplicate to appropriate wells.

> ***Histamine AchE Tracer***

Dispense 100 µL to each well, **except** Blank (Bk) wells.

Incubating the plate

Cover the plate with the cover sheet and incubate 24 hours at +4°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 5 times with 300 µL 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 well. Cover the plate with an aluminium foil sheet and incubate in the

Example Version

dark at room temperature on 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 0.2 – 0.8 A.U. (blank subtracted).**

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
Steps	Blank	Maximum binding	Standard, QC & sample
Derivatization	-	200 µL of assay medium	200 µL of standard, QC or sample
	-	20 µL of 1.5M NaOH if assay medium is $HClO_4$	
	-	50 µL of derivatization agent	
	Vortex all tubes		
	-	20 µL of derivatization agent + immediate vortexing of each tube	
Wash the plate 5 times			
Distribution of reagents	-	100 µL of derivatized solution	
	-	100 µL of Tracer	
Cover plate, incubate at +4°C for 24 hours			
Wash strips 5 times & remove the liquid from the wells			
Developing	200 µL of Ellman's Reagent		
Incubate with an orbital shaker in the dark at room temperature			
Read the plate between 405 and 414 nm			

Example Version

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 at this step.

- Calculate the average absorbance for B0, standard, quality control and sample wells.
- Calculate the B/B0 (%) for each standard, QC and sample (average absorbance of standards, QC or sample divided by average absorbance of B0) & multiply by 100.
- Using a semi-log graph paper, for each standard point, plot 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 sample, the corresponding B/B0 (%) value has to fall within the linear range of the standard curve (usually comprised between 20% and 80%). Find the B/B0 (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Samples which concentration determined on standard curve is greater than 50 nM should be re-assayed after appropriated dilution in Assay buffer prior to the derivatization step.

Example Version

- 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 (see the label of the QC vial)

Acceptable range

- B0 absorbance > 200 mA.U.
- IC50: 3,0 – 4,5 nM
- QC sample: ± 25% of the expected concentration (see the label of QC vial)

Example Version

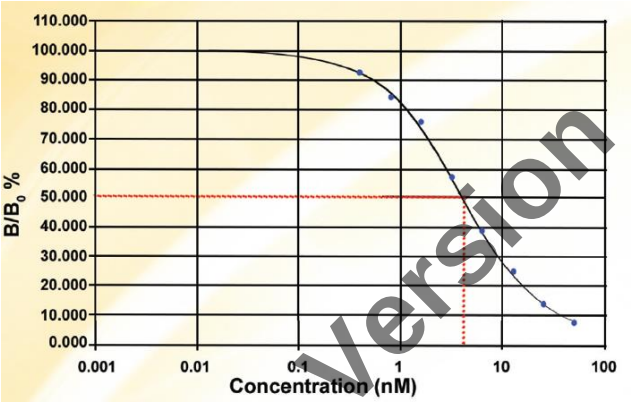
Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.
The 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 4-parameter curve fitting was used to determine the concentrations.

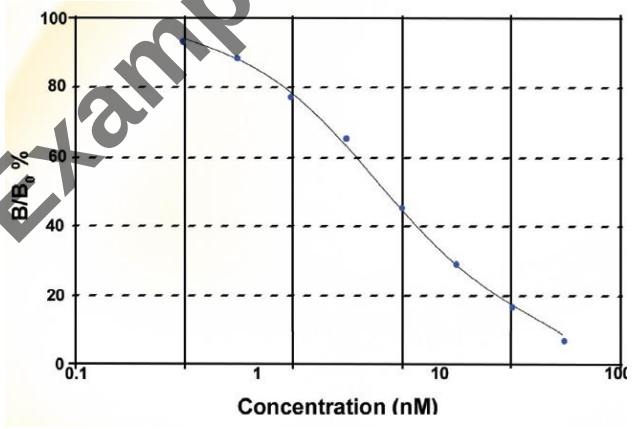
Standard or QC	Histamine ELISA Buffer		Cell culture medium (RPMI)		Perchloric acid ($HClO_4$)	
	mA.U.	B/B0 (%)	mA.U.	B/B0 (%)	mA.U.	B/B0 (%)
B0	515	100,0	514	100,0	321	100,0
50 nM	30	5,8	45	8,8	21	6,5
25 nM	65	12,6	83	16,1	52	16,2
12.5 nM	124	24,1	148	28,8	92	28,7
6.25 nM	215	41,7	231	44,9	145	45,2
3.13 nM	329	63,9	323	62,8	210	65,4
1.56 nM	446	86,6	399	77,6	248	77,3
0.78 nM	499	96,9	414	80,5	285	88,8
0.39 nM	515	100,0	453	88,1	300	93,5
QC 4 nM	249	48,3		48,4	-	77,6

Example version

Standard curve in Histamine ELISA buffer:

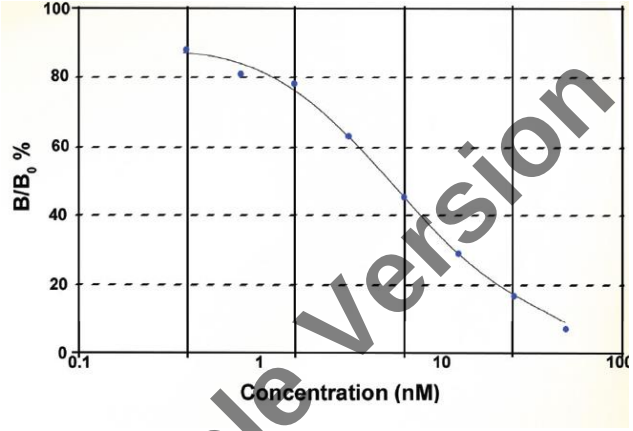


Standard curve in perchloric acid (HClO4):



Example Version

Standard curve in cell culture medium (RPMI):



Example Version

Troubleshooting

Absorbance values are too low:

- organic contamination of water
- one reagent has not been dispersed
- incorrect preparation / dilution
- assay performed before reagents reached room temperature
- reading time not long enough
- DMF used for derivatization was not fresh

> ***High signal and background in all wells:***

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

> ***High dispersion of duplicates:***

- poor pipetting technique
- irregular plate washing

> ***IC_{50} or QC concentrations not within expected range:***

- wrong preparation of standards

> ***Analysis of two dilutions of a biological sample are not coherent***

- interfering substances are present. Sample must be purified prior to ELISA 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.

Example Version

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 ELISA Training kit #B05005. Feel free to contact our Technical Support. We are always happy to hearing from you.

Example Version

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 enzymo-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

4. Valentin MA., Ma S, Zhao A., Legay F., Avrameas A.
Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies.
J Pharm Biomed Anal. (2011) 55(5) : 869-877

5. European Medicines Agency
Guideline on bioanalytical method validation, 21 July 2011

6. Kassas-Guediri A., Coudrat J., Pacreau E. *et al.*
Phospholipid scramblase 1 amplifi es anaphylactic reactions *in vivo*.
PLoS One. 2017 Mar 10 ;12(3) : e0173815

7. Shimoda T., Obase Y., Matsuse H. *et al.*
The Pathogenesis of Alcohol-Induced Airfl ow Limitation in Acetaldehyde Dehydrogenase 2-Defi cient Mice.
Int Arch Allergy Immunol. 2016 ; 171(3-4) :276-284

8. Choi H., Masaru Tanaka M. and Sugimoto K.
Difference in Cell Proliferation and Spontaneous Mediator Release Between Two Mast Cell Lines, NCL-2 and RBL-2H3 on Honey Comb-like Structured Film.

Example Version

J Nanotechnol Nanomed Nanobiotechnol 2016, 3: 009

9. Isaka M., Befu M., Matsubara N. *et al.*
Histamine concentration is involved in canine valvular disease.
Veterinary Science Development 2014 ; 4 :5123

10. Graf A. *et al.*
Knockout of histidine decarboxylase decreases bile duct ligation induced biliary hyperplasia via downregulation of the histidine decarboxylase/VEGF axis through PKA-ERK1/2 signaling
Am J Physiol Gastrointest Liver Physiol 307: G813–G823, 2014.

11. Saligrama N. et *al.*
Systemic Lack of Canonical Histamine Receptor Signaling Results in Increased Resistance to Autoimmune Encephalomyelitis
J Immunol 2013; 191:614-622

12. Shiraishi Y. et *al.*
Sequential Engagement of Fc«RI on Mast Cells and Basophil Histamine H4 Receptor and Fc«RI in Allergic Rhinitis
J Immunol 2013; 190:539-548

13. Youngmi K. *et al.*
Histone Deacetylase 3 Mediates Allergic Skin Inflammation by Regulating Expression of MCP1 Protein*
J. Biol. Chem. 2012, 287:25844-25859.

14. Skovgaard N. *et al.*
Histamine induces postprandial tachycardia through a direct effect on cardiac H2-receptors in pythons
Am J Physiol Regulatory Integrative Comp Physiol 296:774-785, 2009.

15. Yanagisawa R. et *al.*
Titanium Dioxide Nanoparticles Aggravate Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice
Exp Biol Med 234:314–322, 2009

16. Nautiyal K. *et al.*
Brain mast cells link the immune system to anxiety-like behavior
PNAS (2008) 10.1073/pnas.0809479105 Supporting Information

Example Version

17. Struthers S.*et al.*
Pharmacological Characterization of a Novel Nonpeptide Antagonist of the Human Gonadotropin-Releasing Hormone Receptor, NBI-42902
Endocrinology 2007 148:857-867

18. Noguchi J et *al.*
Strain difference of murine bone marrow-derived mast cell functions
J. Leukoc. Biol. 78:
605–611; 2005

19. Furutani K et *al.*
Crucial Role of Histamine for Regulation of Gastric Acid Secretion Ascertained by Histidine Decarboxylase-Knockout Mice
JPET
307:331–338, 2003

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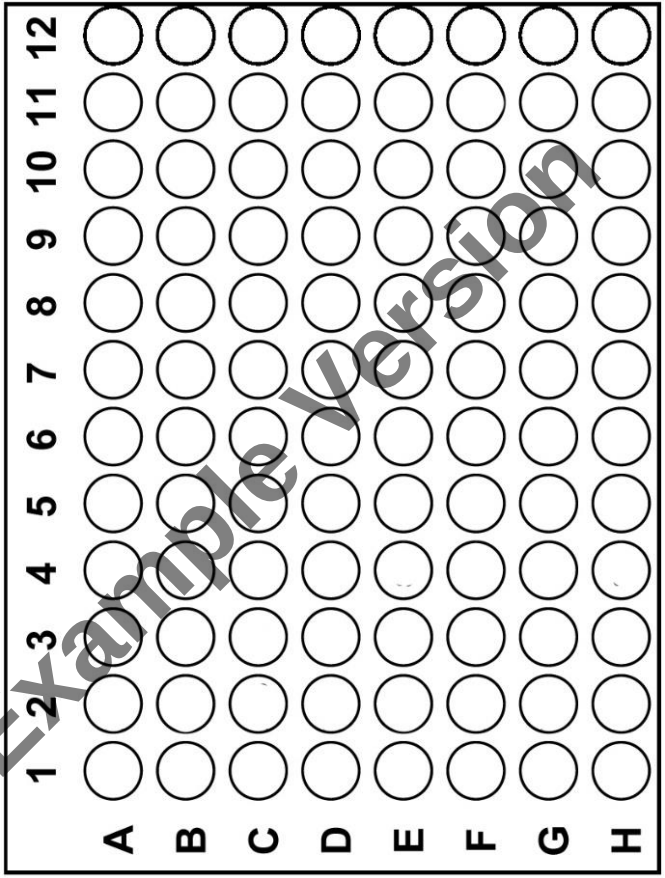

Example Version

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
Should you need help with an order, you can contact our customer service by emailing to order@bertin-bioreagent.com

Please visit our websites:
Bertin-technologies.com
Bertin-Bioreagent.com
Bertin-Corp.com

Example Version

bertin bioreagent

CONTACT US

Bertin Technologies

10 bis Avenue Ampère

Parc d'Activités du Pas du Lac 78180

Montigny-le-Bretonneux

+33 (0)139 306 036

tech@bertin-bioreagent.com

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

Example Version