CXCL10 (pig)

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

CXCL10 (pig)

Enzyme Immunoassay kit

#A05419.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11419
Version: 0119

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
CXCL10 precoated 96-well Strip Plate	Blister with zip	A08419.1 ea	1	-
Streptavidin Poly_HRP Tracer	Green	A04410.100 dtn	1	Liquid
CXCL10 (pig) Biotin-labelled Antibody	Red	A03419.100 dtn	1	Liquid
CXCL10 (pig) Standard	Gold	A06419.1 ea	2	Lyophilised
Poly_HRP EIA Buffer	Grey / Blue	A07410.1 ea	1	Lyophilised
Wash Buffer	Silver	A17000.1 ea	1	Liquid
Tween 20	Transparent	A12000.1 ea	1	Liquid
HRP Substrate Solution	Black	A09034.100 dtn	1	Liquid
HRP Stop Solution	Yellow	A22410.100 dtn	1	Liquid
Technical Booklet	-	A11419.1 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 37 samples in duplicate. If you want to use the kit in two times, we provide one additional vial of Standard.

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

HRP Stop Solution and HRP Substrate Solution are harmful solutions. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

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

CXCL10 is also known as Interferon gamma-induced protein 10 (IP-10) or small-inducible cytokine B10 with a molecular weight close to 10 kDa.

CXCL10 is expressed by several cellular types like monocytes, endothelial cells and fi broblasts [1] stimulated by the interferon-gamma (INF-γ).

The receptor of this chimiokine is CXCR3 [2] that is shared with CXCL9 and CXCL11 [3].

CXCL-10 plays an important role in the recruitment of the immune cells to sites of infl ammation [3]. It is included in physiological process like bone marrow colony formation and angiogenesis [3, 6] but also in some disorders like Th1-type human inflammatory diseases [4], or cancer.

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to CXCL10 (pig).

CXCL10 (pig) introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a second polyclonal antibody tagged with biotin also specific for CXCL10 (pig).

The two antibodies then form a sandwich by binding on

different parts of the CXCL10 (pig).

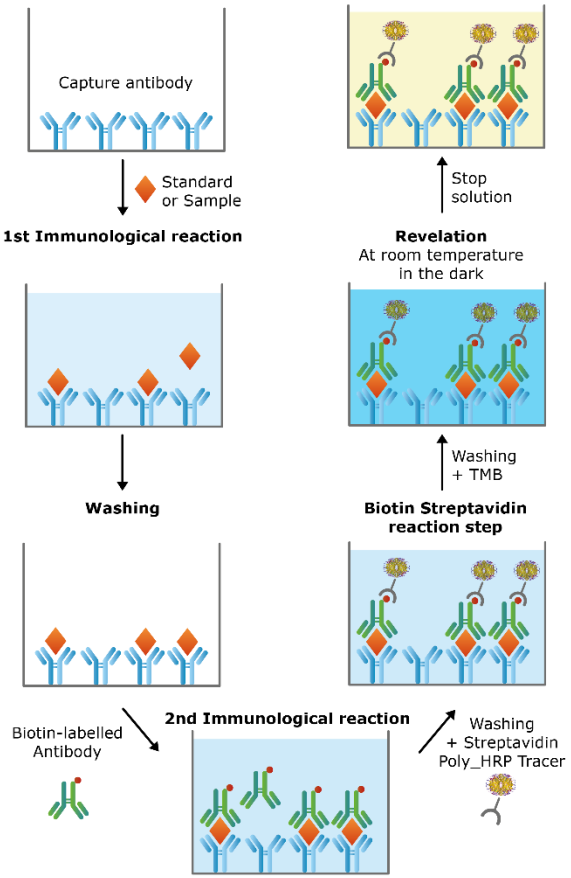
The sandwich is immobilised on the plate so reagents in excess may be washed away.

The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The concentration of CXCL10 (pig) is determined by measuring the enzymatic activity of immobilized Tracer using TMB. The Tracer acts on TMB to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry at 450 nm, is proportional to the amount of CXCL10 (pig) present in the well during the immunological incubation.

The principle of the assay is summarised below:



Assay characteristics

All data shown below are from experiments realized in Buffer. No validation in biological matrix.

- ***Limit of detection (LOD):*** ≤0.08 ng/mL (calculated as the concentration of CXCL10 corresponding to the NSB average plus three standard deviations)

- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
Recombinant (bovine) CXCL10	None
Recombinant (canine) CXCL10	None
Recombinant (equine) CXCL10	None
Recombinant (feline) CXCL10	None
Recombinant (human) CXCL10	None
Recombinant (mouse) CXCL10	None
Recombinant (ovine) CXCL10	None
Recombinant (rabbit) CXCL10	None
Recombinant (rat) CXCL10	None

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 (450 nm filter)
- Microplate washer (or washbottles)
- 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 may be used to measure CXCL10 (pig).

It is the responsibility of the user to check the compatibility of the assay with the study matrix.

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.

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 37 samples in duplicate.

If you want to use the kit in two times, we provide one additional vial of Standard.

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

Poly_HRP EIA Buffer

Reconstitute the Poly_HRP EIA Buffer #A07410 with 25 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.

Before use, filter the Buffer on 0,22 µm filter.

CXCL10 (pig) Standard

Reconstitute the CXCL10 (pig) Standard vial #A06419 with 1 mL of UltraPure water. Allow it to stand for 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

The concentration of the first standard (S1) is 10.00 ng/mL. Prepare seven polypropylene tubes (for the seven other standards) and add 500 µL of Poly_HRP EIA Buffer into each tube. Then prepare the standards by serial dilutions as follow:

Standard	Volume of Standard	Volume of Poly_HRP EIA Buffer	Standard concentration ng/mL
S1	-	-	10.00
S2	500 µL of S1	500 µL	5.00
S3	500 µL of S2	500 µL	2.50
S4	500 µL of S3	500 µL	1.25
S5	500 µL of S4	500 µL	0.63
S6	500 µL of S5	500 µL	0.31
S7	500 µL of S6	500 µL	0.16
S8	500 µL of S7	500 µL	0.08

Stability at 4°C: within the day

CXCL10 (pig) Biotin-labelled Antibody

The supplied CXCL10 (pig) Biotin-labelled Antibody is concentrated 10 times. Calculate the volume needed (number of wells multiplied by 0.1 mL) and then dilute the

CXCL10 (pig) Biotin-labelled Antibody solution #A03419 with the appropriate volume of Poly_HRP EIA Buffer.

Example: *for 40 wells you need 4 mL of CXCL10 (pig) Biotin-labelled Antibody (40 x 0.1 mL), add 0.4 mL of CXCL10 (pig) Biotin-labelled Antibody in 3.6 mL of Poly_HRP EIA Buffer.*

Stability of diluted antibody at +4°C: within the day.

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.

Stability at +4°C: 1 month.

Assay procedure

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

Plate preparation

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

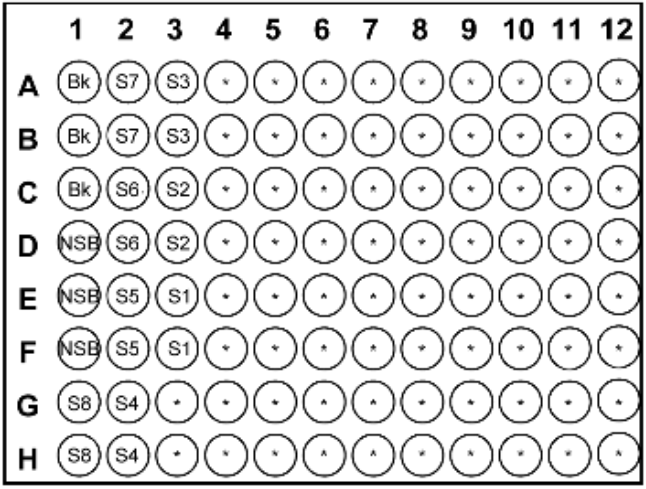
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.

Rinse each well 5 times with 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
S1-S8 : Standards 1-8
* : Samples

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet the 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.

Poly_HRP EIA Buffer

Dispense 100 µL to Non Specific Binding wells (NSB) wells.

CXCL10 (pig) Standard

Dispense 100 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.

CXCL10 (pig) Samples

Dispense 100 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in Poly_HRP EIA Buffer.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an

orbital microplate shaker.

Washing the plate

Rinse each well 5 times with Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

- ***CXCL10 (pig) Biotin-labelled antibody***

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

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 5 times with Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

- ***Streptavidin Poly_HRP Tracer***

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

Incubating the plate

Cover the plate with the cover sheet and incubate 30 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Developing and reading the plate

- Empty the plate by turning it over. Rinse each well 5 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 100µL of HRP Substrate Solution to each well.
- Incubate the plate in the dark at room temperature without shaking. For the time, look at the lot specific Quality Control Sheet (QCS). In general, revelation time is 10 min.
- Add 100µL of HRP Stop Solution to each well.
- 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 450 nm (yellow color).

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)				
	Blank	NSB	Standard	Sample
Poly_HRP EIA Buffer	-	100	-	-
Standard	-	-	100	-
Sample	-	-	-	100
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
Biotin-labelled Antibody	-	100		
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
Streptavidin Poly_HRP Tracer	-	100		
Cover plate, incubate **30** minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 µL/well Discard liquid from the wells & dry on absorbent paper				
HRP Substrate Solution	100			
Incubate the plate in the dark without agitation				
HRP Stop Solution	100			
Read the plate at 450 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. If not, do it at this steps.

- Calculate the average absorbance for each NSB, standard and sample.
- For each standard, plot the absorbance 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 absorbance 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 10.00 ng/mL should be re-assayed after dilution in Poly_HRP EIA Buffer.
- Most plate readers are supplied with a curve-fitting software capable of graphing these data (4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Acceptable range

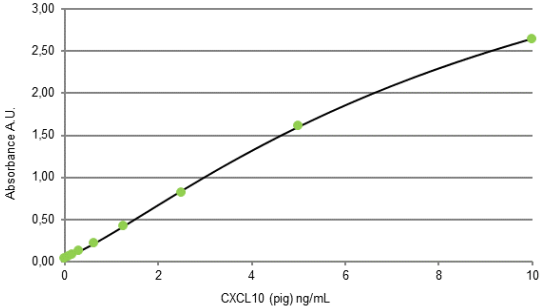
- NSB absorbance ≤ 0.100 A.U.
- Limit of detection ≤ 0.08 ng/mL

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: 10 minutes developing at room temperature, reading at 450 nm. A 4 parameter logistic fitting was used to determine the concentrations.

Standard	CXCL10 (pig) ng/mL	Absorbance A.U.
S1	10.00	2.645
S2	5.00	1.613
S3	2.50	0.821
S4	1.25	0.425
S5	0.63	0.223
S6	0.31	0.129
S7	0.16	0.088
S8	0.08	0.067
NSB	0.00	0.044

Typical CXCL10 (pig) standard curve



Troubleshooting

Absorbance values are too low:

- one reagent has not been 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 technique
- irregular plate washing.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent 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 tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Technical Support.

Bibliography

1. Luster A. D., Unkeless J. C., Ravetch J. V.
Gamma-interferon transcriptionally regulates an early-response gene containing homology to platelet proteins.
1985 Nature 315:672.

2. Booth V,. Keizer D.W., Kamphuis M.B., et al.
The CXCR3 binding chemokine IP-10/CXCL10: structure and receptor interactions.
2002 Biochemistry. 41 (33): 10418–25.

3. Dufour J. H., Dziejman M., Liu M. T., et al.
IFN-γ-Inducible Protein 10 (IP-10; CXCL10)-Deficient Mice Reveal a Role for IP-10 in Effector T Cell Generation and Trafficking.
2002 J Immunol 168:3195-3204

4. Gottlieb, A. B., Luster A. D., Posnett D. N., et al.
Detection of a γ-interferon-induced protein IP-10 in psoriatic plaques.
1988 J. Exp. Med. 168:941-948.

5. Angiolillo A.L., Sgadari C., Taub D.D., et al.
Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis in vivo.
1995 The Journal of Experimental Medicine. 182 (1): 155–162.

1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

4

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 Instruments offers a range of unique laboratory equipment from Air Sample collection, Sample Homogenisation and Digital Imaging.

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-instruments.com
Bertin-Bioreagent.com
Bertin-Corp.com

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