



## CCL2 (pig)

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**CCL2 (pig)**  
**Enzyme Immunoassay kit**  
**#A05415.96 wells**

For research laboratory use only  
Not for human diagnostic use

This assay was developed  
& validated by Bertin Bioreagent

Fabriqu  en France  
Made in France



#A11415  
Version: 0118

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

This kit contains:

<b>Designation</b>	<b>Colour of cap</b>	<b>Item #</b>	<b>Quantity per kit</b>	<b>Form</b>
CCL2 precoated 96-well Strip Plate	Blister with zip	A08415.1 ea	1	-
Streptavidin Poly_HRP Tracer	Green	A04410.100 dtn	1	Liquid
CCL2 (pig) Biotin- labelled Antibody	Red	A03415.100 dtn	1	Liquid
CCL2 (pig) Standard	Blue with red septum	A06415.1 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	-	A11415.1 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.

## ► **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

CCL2 also known as the monocyte chemoattractant protein-1 (MCP-1) is a chemokine and more particularly a C-C chemokine.

Initially discovered in 1983, it is a monomeric polypeptide of 73 amino acids (13 kDa) [1]. It belongs to a family composed at least of members MCP-1, -2, -3, and -4.

CCL-2 is produced by the immune cells [2] (monocyte/macrophages) and non-immune cells (endothelial cells, fibroblastes, myocytes) [3].

The main function of the CCL2 is the chemotactism and more particularly the regulation of the migration and the infiltration of monocytes, memory T lymphocytes [4], and natural killer (NK) cells on an infection site. CCL2 is involved in many diseases like HIV-1 pathogenesis, cancer, cardiovascular disease or diabetes [5].

## ► Principle of the assay

This Enzyme Immuno-Assay (EIA/ELISA) is based on a sandwich technique. Wells of supplied plate are coated with a polyclonal antibody specific to CCL2 (pig).

CCL2 (pig) introduced into the wells (standard or sample) is bound by the polyclonal antibody coated on the plate and is then detected by another polyclonal antibody tagged with biotin also specific for CCL2 (pig).

The two antibodies then form a sandwich by binding on different epitopes of the CCL2 (pig).

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

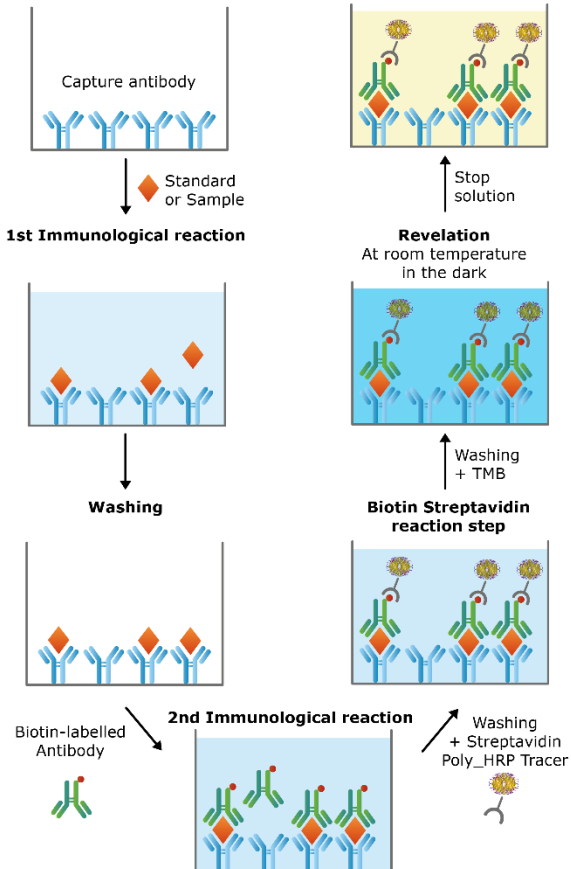
The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The concentration of CCL2 (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 CCL2 (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.

- > **Limit of detection (LOD):**  $\leq 3.9$  pg/mL (calculated as the concentration of CCL2 (pig) corresponding to the NSB average plus three standard deviations)

### > Cross-reactivity

Species	Cross-reactivity
Recombinant CCL2 (bovine)	+++
Recombinant CCL2 (equine)	+++
Recombinant CCL2 (canine)	+++
Recombinant CCL2 (guinea pig)	+/-
Recombinant CCL2 (mouse)	+/-
Recombinant CCL2 (rabbit)	-

## ► Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

- Precision micropipettes (20 to 1000  $\mu$ L)
- Spectrophotometer plate reader (450 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300 $\mu$ L

- 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 CCL2 (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.

## ► 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 according to suggested plate layout.

An additional vial of Standard is provided in case you need to perform 2 assays with the kit.

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.

### ► CCL2 (pig) Standard

Reconstitute the CCL2 (pig) Standard vial #A06415 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 500 pg/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
S1	-	-	500.0 pg/mL
S2	500 µL of S1	500 µL	250.0 pg/mL
S3	500 µL of S2	500 µL	125.0 pg/mL
S4	500 µL of S3	500 µL	62,5 pg/mL
S5	500 µL of S4	500 µL	31.3 pg/mL
S6	500 µL of S5	500 µL	15.6 pg/mL
S7	500 µL of S6	500 µL	7.8 pg/mL
S8	500 µL of S7	500 µL	3.9 pg/mL

*Stability at 4°C: within the day.*

### ► **CCL2 (pig) Biotin-labelled Antibody**

The supplied CCL2 (pig) Biotin-labelled Antibody is concentrated 10 times. Calculate the volume needed (number of wells multiplied by 0.1 mL) and then dilute the CCL2 (pig) Biotin-labelled Antibody solution #A03415 with the appropriate volume of Poly\_HRP EIA Buffer.

Example: for 40 wells you need 4 mL of CCL2 (pig) Biotin-labelled Antibody (40 x 0.1 mL), add 0.4 mL of CCL2 (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  $\mu$ L 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  $\mu$ L/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.

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

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.

> ***CCL2 (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.

> ***CCL2 (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**

➤ ***CCL2 (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  $\mu$ L 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 100 $\mu$ L 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 100 $\mu$ L 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 $\mu\text{L}$ )				
	Blank	NSB	Standard	Sample
Poly_HRP EIA Buffer	-	100	-	-
Standard	-	-	100	-
Sample	-	-	-	100
Cover plate, incubate <b>60</b> minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 $\mu\text{L}$ /well				
Discard liquid from the wells & dry on absorbent paper				
Biotin-labelled Antibody	-	100		
Cover plate, incubate <b>60</b> minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 $\mu\text{L}$ /well				
Discard liquid from the wells & dry on absorbent paper				
Streptavidin Poly_HRP Tracer	-	100		
Cover plate, incubate <b>30</b> minutes at room temperature under orbital shaking at 300 rpm				
Wash strips 5 times with 300 $\mu\text{L}$ /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 500 pg/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  $\leq 0.060$  A.U.
- Limit of detection  $\leq 3.9$  pg/mL

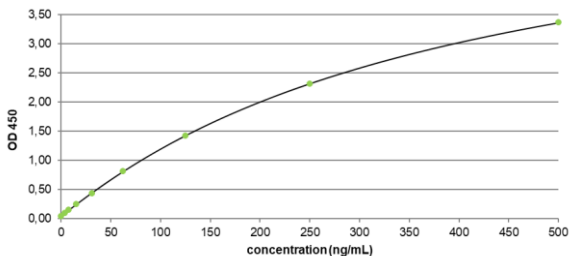
## ► **Typical results**

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 with a 1/Y ponderation was used to determine the concentrations.

Standard	CCL2 (pig) pg/mL	Absorbance A.U.
S1	500.0	3.360
S2	250.0	2.311
S3	125.0	1.421
S4	62.5	0.809
S5	31.3	0.428
S6	15.6	0.245
S7	7.8	0.147
S8	3.9	0.0897
NSB	0.00	0.039

Typical CCL2 (pig) standard curve



## ► Troubleshooting

### ➤ **Absorbance values are too low:**

- organic contamination of water,
- 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](mailto: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. For further information, please contact our Technical Support.

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





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.

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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](mailto:tech@bertin-bioreagent.com)

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