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MIP-1β (pig)

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MIP-1β (pig)

Enzyme Immunoassay kit

#A05417.96 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

#A11417
Version: 0118

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
MIP-1β (pig) precoated 96-well Strip Plate	Blister with zip	A08417.1 ea	1	-
Low Streptavidin Poly_HRP Tracer	Green	A04417.100 dtn	1	Liquid
MIP-1β (pig) Biotin-labelled Antibody	Red	A03417.100 dtn	1	Liquid
MIP-1β (pig) Standard	Blue with red septum	A06417.1 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	-	A11417.1 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

Macrophage inflammation protein-1β (MIP-1β), also known as CCL4, is a C-C Chemokine produced by macrophages after stimulation by bacterial endotoxins[1]. This chemokine is crucial for immune responses towards infection and inflammation[2]. It is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells[3].

When produced by CD8+ T cells, MIP-1β is also a major HIV-suppressive factor[4].

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 MIP-1β (pig).

MIP-1β (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 MIP-1β (pig).

The two antibodies then form a sandwich by binding on different parts of the MIP-1β (pig).

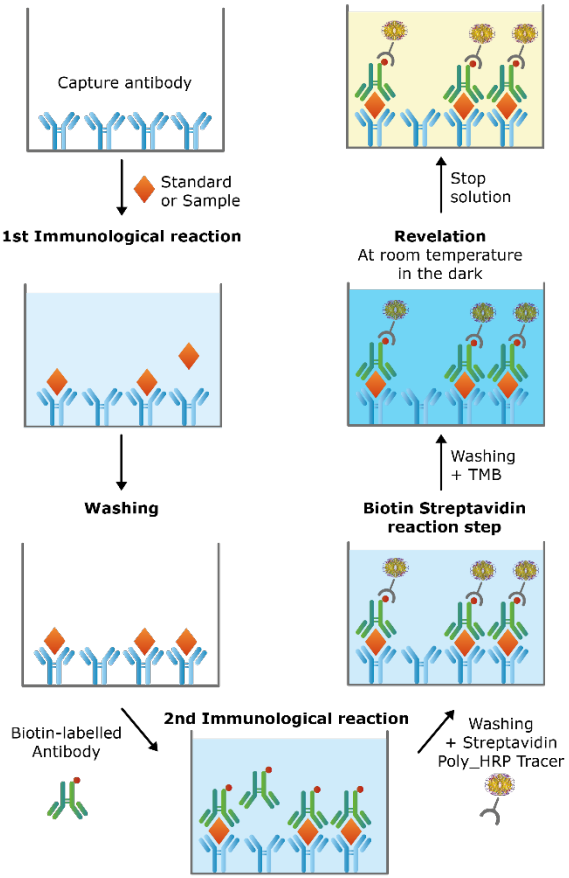
The sandwich is immobilised on the plate so reagents in excess may be washed away.

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

The concentration of MIP-1β (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 MIP-1β (pig) present in the well during the immunological incubation.

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 (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 MIP-1β (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.

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.

MIP-1β (pig) Standard

Reconstitute the MIP-1β (pig) Standard vial #A06417 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 2.00 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
S1	-	-	2.00 ng/mL
S2	500 µL of S1	500 µL	1.00 ng/mL
S3	500 µL of S2	500 µL	0.50 ng/mL
S4	500 µL of S3	500 µL	0.25 ng/mL
S5	500 µL of S4	500 µL	0.13 ng/mL
S6	500 µL of S5	500 µL	0.06 ng/mL
S7	500 µL of S6	500 µL	0.03 ng/mL
S8	500 µL of S7	500 µL	0.02 ng/mL

Stability at 4°C: within the day.

MIP-1β (pig) Biotin-labelled Antibody

The supplied MIP-1β (pig) Biotin-labelled Antibody is concentrated 10 times. Calculate the volume needed (number of wells multiplied by 0.1 mL) and then dilute the MIP-1β (pig) Biotin-labelled Antibody solution #A03417 with the appropriate volume of Poly_HRP EIA Buffer.

Example: for 40 wells you need 4 mL of MIP-1β (pig) Biotin-labelled Antibody (40 x 0.1 mL), add 0.4 mL of MIP-1β (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.

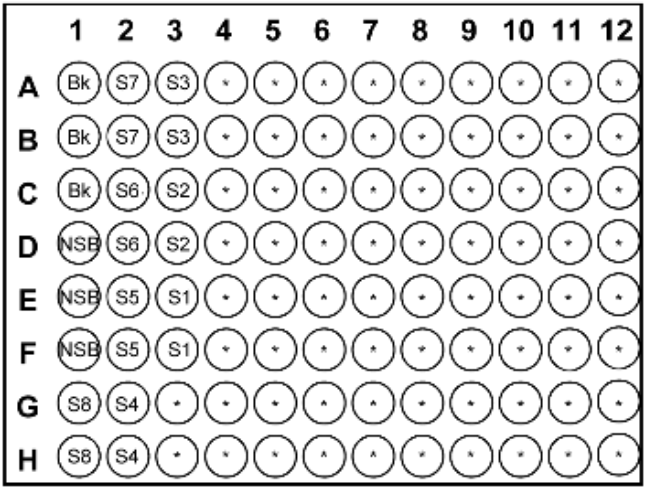
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet.
Stability at +4°C: 1 month.

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.

MIP-1β (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.

MIP-1β (pig) Sample

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.

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

> ***MIP-1β (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.

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

> ***Low 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.

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).

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				
Low 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 now.

- 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 2.00 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

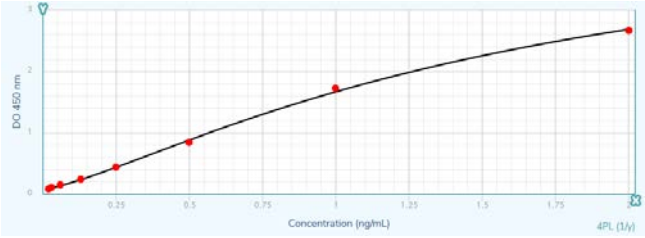
- NSB absorbance ≤ 0.100 A.U.
- Limit of detection ≤ 0.02 ng/mL

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

Standard	MIP-1β (pig) ng/mL	Absorbance A.U.
S1	2.00	2.667
S2	1.00	1.722
S3	0.50	0.841
S4	0.25	0.435
S5	0.13	0.238
S6	0.06	0.147
S7	0.03	0.104
S8	0.02	0.078
NSB	0.00	0.061

Typical MIP-1β (pig) standard curve



Characteristics

- ***Limit of detection*** calculated as the concentration of MIP-1β (pig) corresponding to the NSB average plus three standard deviations is ≤0.02 ng/mL.

- ***Cross-reactivity***

Species	Cross-reactivity
Recombinant MIP-1β (bovine)	Weak
Recombinant MIP-1β (canine)	None
Recombinant MIP-1β (chicken)	None
Recombinant MIP-1β (equine)	Strong
Recombinant MIP-1β (human)	Weak
Recombinant MIP-1β (mouse)	None
Recombinant MIP-1β (rabbit)	None
Recombinant MIP-1β (rat)	None

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. Sherry B, Tekamp-Olson P, Gallegos C *et al.* *Resolution of the two components of macrophage inflammatory protein 1, and cloning and characterization of one of those components, macrophage inflammatory protein 1 beta.* December 1988 J. Exp. Med. 168 (6): 2251–9

2. Ren M, Guo Q, Guo L, *et al.* *Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme*. December 2010 EMBO J. 29 (23): 3952–66

3. Bystry RS, Aluvihare V, Welch KA *et al.* *B cells and professional APCs recruit regulatory T cells via CCL4.* December 2001 Nat. Immunol. 2 (12): 1126–32

4. Cocchi F, DeVico AL, Garzino-Demo A *et al.* *Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells.* December 1995 Science. 270 (5243): 1811–5

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Bertin Bioreagent, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and biochemicals thanks to its innovative work in research and development.

Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

Bertin Bioreagent is active worldwide either with direct sales or through our qualified and trained international distribution network from the United States to Japan.

We are able to provide you with local technical support to use at ease our products.

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