

## Rat CCL4 ELISA Kit

**Cat#: orb106608 (ELISA Manual)**

### Assay Principle

The Biorbyt Rat Ccl4 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Rat Ccl4 with a 96-well strip plate that is pre-coated with antibody specific for Ccl4. The detection antibody is a biotinylated antibody specific for Ccl4. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Rat Ccl4 with immunogen: Expression system for standard: E.Coli; Immunogen sequence: A24-N92. The kit is analytically validated with ready to use reagents. To measure Rat Ccl4, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Rat Ccl4 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Rat Ccl4 in the sample.

### Overview

Product Name Rat CCL4/MIP-1 Beta ELISA Kit

Reactive Species Rat

Size 96wells/kit, with removable strips.

Description Sandwich High Sensitivity ELISA kit for Quantitative Detection of Rat CCL4/MIP-1 beta. 96wells/kit, with removable strips.

Sensitivity <10pg/ml

\*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

Detection Range 15.6pg/ml-1000pg/ml

Storage Instructions Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw Cycles (Shipped with wet ice.)

Uniprot ID P50230

### Technical Details

Capture/Detection Antibodies The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.

Specificity Natural and recombinant Rat Ccl4

Immunogen Expression system for standard: E.Coli; Immunogen sequence: A24-N92  
 Cross Reactivity There is no detectable cross-reactivity with other relevant proteins.

### Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

### Kit Components/Materials Provided

Description	Quantity	Volume
Anti-Rat Ccl4 Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Rat Ccl4 Standard	2	10ng/tube
Rat Ccl4 Biotinylated antibody (100x)	1	130 µl
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µl
Sample Diluent	1	30ml
Antibody Diluent	1	12ml
Avidin-Biotin-Peroxidase Diluent	1	12ml
Color Developing Reagent (TMB)	1	10ml
Stop Solution	1	10ml
Plate Sealers	4	Piece
Wash Buffer	1	Powder pack for 1000ml

### Required Materials That Are Not Supplied

- Microplate Reader capable of reading absorbance at 450nm.
- Automated plate washer (optional)
- Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
- Deionized or distilled water.
- 500ml graduated cylinders.
- Test tubes for dilution.
- Washing buffer (neutral PBS or TBS).

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450?l of purified acetic acid or 700?l of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O

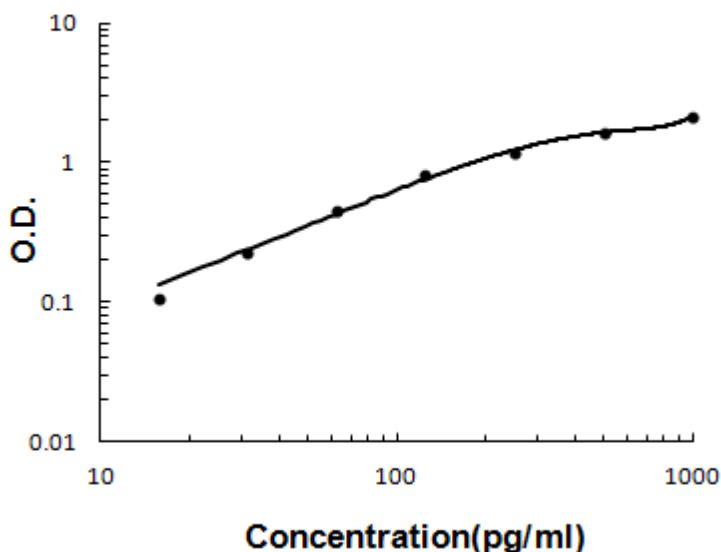
Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na O and adjust pH to 7.2-7.4

### Rat CCL4/MIP-1 Beta ELISA Kit Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	15.6	31.2	62.5	125	250	500	1000
O.D.	0.005	0.112	0.234	0.450	0.835	1.179	1.667	2.174

Rat CCL4/MIP-1 beta ELISA Kit standard curve  
**Rat CCL4 ELISA Kit**



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### Intra/Inter Assay Variability

Biorbyt spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

**Intra-Assay Precision (Precision within an assay):** Three samples of known concentration were tested on one plate to assess intra-assay precision.

**Inter-Assay Precision (Precision across assays):** Three samples of known concentration were tested in separate assays to assess interassay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	31	144	453	31	148	419
Standard deviation	1.42	9.21	24.31	1.67	12.13	24.3
CV(%)	4.6%	6.4%	5.5%	5.4%	8.2%	5.8%

## Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	31	27	31	27	29	2	6.8%
Sample 2	144	160	147	149	150	6.04	4%
Sample 3	453	458	508	520	484	29.6	6.1%

\*number of samples for each test n=16.

## Preparation Before The Experiment

### All reagents

Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C.

### Wash buffer

Prepare standard 1X PBS as wash buffer. Preparation of wash buffer: Add 8.5g NaCl, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6.

### Biotinylated Anti-Rat Ccl4 antibody

It is recommended to prepare this reagent immediately prior to use by diluting the Rat Ccl4 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

### Avidin-Biotin-Peroxidase Complex

It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin- Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

## Rat Ccl4 Standard

It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Rat Ccl4 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

### Microplate

The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

### Dilution of Rat Ccl4 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –1000pg/ml, #2 –500pg/ml, #3 – 250pg/ml, #4 – 125pg/ml, #5 – 62.5pg/ml, #6 –31.25pg/ml, #7 – 15.625pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).
2. To generate standard #1, add 100µl of the reconstituted standard stock solution of 10ng/ml and 900µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

### Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

#### Cell culture supernatants

Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.

#### Serum

Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

#### Plasma

Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

\*Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

## Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay. It is recommended to prepare 150  $\mu$ l of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

## Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100  $\mu$ l of the standard, samples, or control per well. Add 100  $\mu$ l of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100  $\mu$ l of the prepared 1x Biotinylated Anti-Rat Ccl4 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 2 additional times.
9. Add 100  $\mu$ l of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 4 additional times.
11. Add 90  $\mu$ l of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100  $\mu$ l of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

## Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

## Background on Ccl4

Chemokine (C-C motif) ligand 4, also known as CCL4, is a protein which in mouse is encoded by the CCL4 gene. It is a CC chemokine with specificity for CCR5 receptors. It is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells. CCL4 is a major HIV suppressive factor produced by CD8+ T cells. Performing-low memory CD8+ T cells that normally synthesize MIP-1-beta. By analysis of somatic cell hybrids and by in situ hybridization, the CCL4 gene was assigned to 17q21-q23. Based on functional analysis of MIP-1beta, one or more additional beta chemokine receptors that transduce MIP-1beta signals must also exist. MIP-1alpha and MIP-1beta have been shown to lack affinity for the erythrocyte surface chemokine receptor/Duffy antigen that binds many alpha as well as beta chemokines (11, 14).