

Mouse CCL22 Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse CCL22 in serum, plasma and cell culture supernatants samples.

General Information

| | |
|---------------------------|--------------------------------|
| Catalogue Number | KE10053 |
| Product Name | Mouse CCL22 Sandwich ELISA Kit |
| Species cross-reactivity | Mouse |
| Range (calibration Range) | 7.8-500 pg/mL |
| Tested applications | Quantification ELISA |

Database Links

| | |
|-------------|--------|
| Entrez Gene | 20299 |
| SwissProt | Q546S6 |

Kit Components & Storage

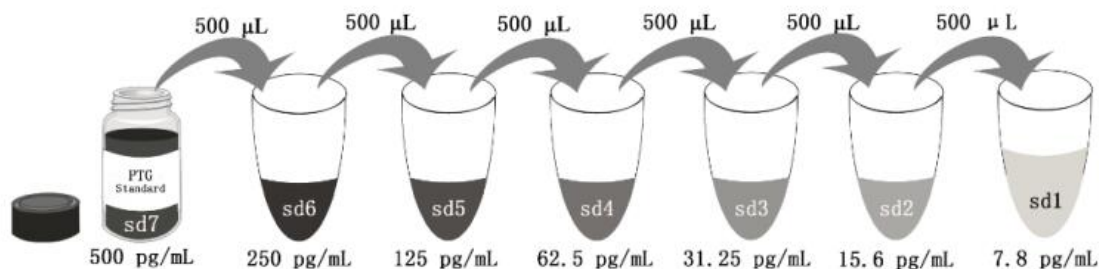
| | | |
|--|-----------|---|
| Microplate - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay. |
| Protein standard - 500 pg/bottle; lyophilized* | 2 bottles | |
| Detection antibody, biotinylated (100X) - 120 µL/vial | 1 vial | |
| Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial | 1 vial | |
| Sample Diluent PT 4-ec - 30 mL/bottle. | 1 bottle | |
| Detection Diluent - 30 mL/bottle | 1 bottle | |
| Wash Buffer Concentrate (20X) - 30 mL/bottle | 1 bottle | |
| Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle | 1 bottle | |
| Stop Solution - 12 mL/bottle | 1 bottle | |
| Plate Cover Seals | 3 pieces | |

NB: Do not use the kit after the expiration date.

Sample Diluent PT 4-ec is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1mL Sample Diluent PT 4-ec in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



| | | | | | | | |
|---|----------------|--------|--------|--------|--------|--------|--------|
| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| # µL of Sample Diluent PT 4-ec | 1000 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

Product Description

KE10053 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CCL22 ELISA kit is to be used to detect and quantify protein levels of endogenous CCL22. The assay recognizes mouse CCL22. An antibody specific for CCL22 has been pre-coated onto the microwells. The CCL22 protein in samples is captured by the coated antibody after incubation.

Following extensive washing, another antibody of biotinylated specific for mouse CCL22 is added to detect the captured mouse CCL22 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

The C-C motif chemokine ligand 22 (CCL22), also known as MDC, belongs to the group of chemokines, that are both constitutively expressed under homeostatic conditions and inducible upon inflammation. CCL22 is a secreted protein that exerts chemotactic activity for monocytes, dendritic cells, natural killer cells, and for chronically activated T lymphocytes. CCL22 is induced by LPS, IL-4, and IL-13 and in T cells by TCR stimulation.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. 1:4 or 1:8 dilution is recommended for serum or plasma, 1:2 or 1:4 dilution is recommended for cell culture supernatants.

Safety Notes

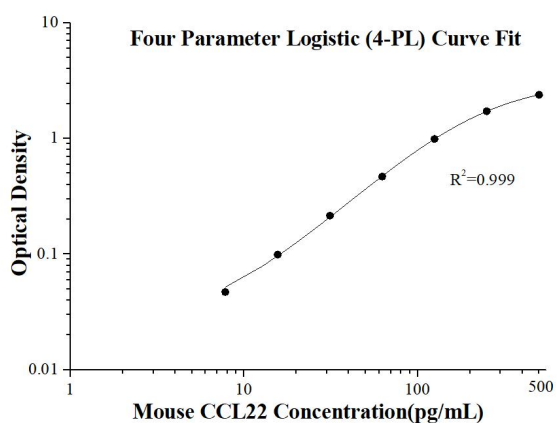
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

| Step | Reagent | Volume | Incubation | Wash | Notes |
|------|--|--------|------------|-------------|------------------------------|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

Example data

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



| (pg/mL) | O.D | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.076 0.073 | 0.075 | - |
| 7.8 | 0.121 0.122 | 0.121 | 0.047 |
| 15.6 | 0.171 0.176 | 0.174 | 0.099 |
| 31.25 | 0.296 0.284 | 0.290 | 0.216 |
| 62.5 | 0.538 0.549 | 0.544 | 0.469 |
| 125 | 1.071 1.057 | 1.064 | 0.990 |
| 250 | 1.801 1.791 | 1.796 | 1.721 |
| 500 | 2.470 2.458 | 2.464 | 2.390 |

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|-----------------------|----|--------------|-----|-----|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 251.2 | 6.7 | 2.7 |
| 2 | 20 | 56.3 | 2.5 | 4.5 |
| 3 | 20 | 12.2 | 1.2 | 9.5 |

| Inter-assay Precision | | | | |
|-----------------------|----|--------------|------|-----|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 24 | 215.9 | 20.9 | 9.7 |
| 2 | 24 | 55.7 | 3.4 | 6.0 |
| 3 | 24 | 12.7 | 1.3 | 9.9 |

Recovery

The recovery of CCL22 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---------------------------|-----|----------------------|-----------|
| Mouse serum | 1:4 | 97 | 85-116 |
| | 1:8 | 108 | 87-121 |
| Cell culture supernatants | 1:4 | 111 | 105-124 |
| | 1:8 | 90 | 83-96 |

Sample Values

Mouse serum samples were evaluated for the presence of mouse CCL22 in this assay.

| Sample Type | Mean (pg/mL) | Range (pg/mL) |
|--------------------|--------------|---------------|
| Mouse serum (n=16) | 234.5 | 120.6-306.8 |

Cell Culture Supernates:

Mouse splenocytes (1×10^6 cells/mL) were cultured for 1 day in RPMI plus 10% fetal bovine serum supplemented with $50 \mu\text{M}$ β -mercaptoethanol and stimulated with $1.0 \mu\text{g/mL}$ LPS. An aliquot of the cell culture supernate was removed, assayed for mouse CCL22.

| Condition | Day 1 (pg/mL) |
|--------------|---------------|
| Unstimulated | 30.2 |
| Stimulated | 84.6 |

Sensitivity

The minimum detectable dose of mouse CCL22 is 0.98 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, mouse serum, cell culture supernatants were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The serum samples were initially diluted 1:2)

| | | Mouse serum | Cell culture supernatants |
|------|----------------------|-------------|---------------------------|
| 1:2 | Average% of Expected | 100 | 100 |
| | Range (%) | - | - |
| 1:4 | Average% of Expected | 106 | 95 |
| | Range (%) | 97-111 | 91-102 |
| 1:8 | Average% of Expected | 109 | 91 |
| | Range (%) | 87-123 | 84-96 |
| 1:16 | Average% of Expected | 109 | - |
| | Range (%) | 101-123 | - |

References

1. A Mantovani. et al. (2000) *J Leukoc Biol.* 68(3):400-4.
2. M Vulcan. et al. (2001) *Eur J Immunol.* 31(3):812-22.
3. Stefanie Scheu. et al. (2017) *Int J Mol Sci.* 18(11):2306
4. Röhrle N. et al. (2020) *Adv Exp Med Biol.* 1231:79-96.