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CCL25
Mouse Chemokine Ligand 25 ELISA Kit

Catalog No: CKM062 **Size:** 1 x 96 wells

Introduction

The Cell Sciences Mouse CCL25 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse CCL25 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse CCL25 coated on a 96-well plate. Standards and samples are pipetted into the wells and CCL25 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse CCL25 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CCL25 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied with the kit:

Items	Quantity
A. CCL25 Microplate coated with anti-mouse CCL25	96 wells (12 strips x 8 wells)
B. Wash Buffer Concentrate (20x)	25 ml
C. Recombinant mouse CCL25 Standards	2 vials
D. Assay Diluent A for Standard/Sample (serum/plasma) diluent*	30 ml
E. Assay Diluent B (5x) for Standard/Sample (cell culture medium) diluent	15 ml
F. Detection Antibody-Biotinylated anti-mouse CCL25	2 vials (each vial is enough to assay half microplate)
G. Streptavidin-HRP Concentrate (6,000x)	8 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 ml
I. Stop Solution (2 M sulfuric acid)	8 ml

* *Contains 0.09% sodium azide as preservative. Precaution: Sodium azide is a poisonous and hazardous substance which should be handled by trained staff only*.

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Storage of Kit Reagents

Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Note: The whole kit is stable for 1 year when stored at -20°C. Avoid repeated freeze-thaw cycles.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Preparation of Kit Reagents

Bring all reagents and samples to room temperature (18 - 25°C) before use.

Sample Dilution

If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of serum/plasma samples, and Assay Diluent B (Item E) for dilution of culture supernatants.

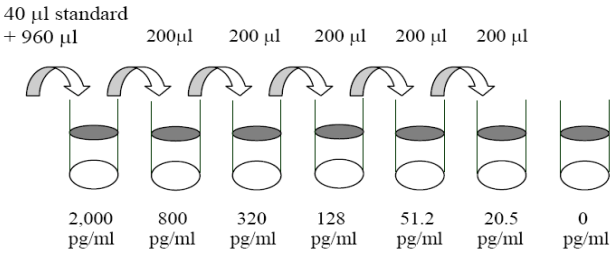
Assay Diluent B

Dilute 5-fold with deionized or distilled water.

CCL25 Standard

- Briefly spin the vial of Item C (Recombinant Mouse CCL25 Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium) into Item C vial to prepare a 50 ng/ml standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 40 µl CCL25 standard from the vial of Item C, into a tube with 960 µl Assay Diluent A or 1x Assay Diluent B to prepare a 2,000 pg/ml stock standard solution.
- Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown on next page in Figure 1).
- Mix each tube thoroughly before the next transfer.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

Figure 1



Wash Buffer Concentrate

- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved.
- Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipette up and down to mix gently (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 65-fold with 1x Assay Diluent B and used in step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and pipette up and down to mix gently before use.
- Streptavidin-HRP concentrate should be diluted 6,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare 6,000 fold diluted HRP-Streptavidin solution.

ELISA Method

Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay.

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: CCL25 Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in Step 3.
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in Step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

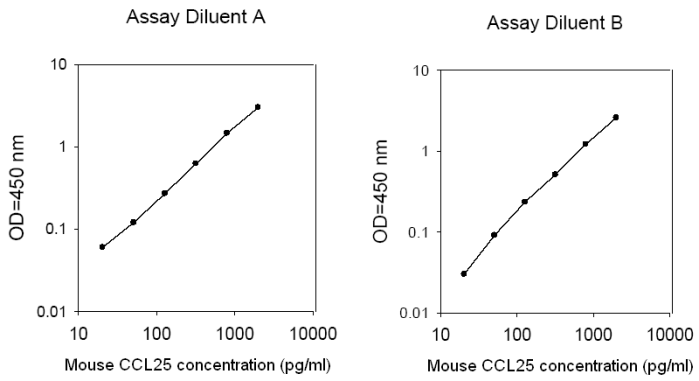
Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Figure 2

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Performances and Characteristics

Sensitivity

The minimum detectable dose of CCL25 is typically less than 15 pg/ml.

Recovery

Recovery was determined by spiking various levels of mouse CCL25 into mouse serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	94.26	82-105
Plasma	92.68	80-106
Cell culture media	97.42	84-107

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	89 81-102	87 80-102	88 82-103
1:4	Average % of Expected Range (%)	95 83-105	93 82-103	94 83-104
1:8	Average % of Expected Range (%)	93 82-104	96 84-106	101 86-109

Reproducibility

Intra-assay: CV<10%

Inter-assay: CV<12%

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, Mouse 6Ckine, CTACK, Eotaxin, GCSF, GM-CSF, IL2, IL3, IL4**,** IL5, IL6, IL9, IL10, IL12 p40, IL12 p70, IL13, IL17, IFN-γ, KC, Leptin, MCP5, MIP1α, MIP2, MIP3β, RANTES, SCF, sTNFri, TARC, TIMP1, TNF-α, Tpo, VEGF).

Troubleshooting Guide

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; ELISA Method Step 2 may change to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage of the ELISA Kit	1. Store your standard at < -20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

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