

Human CCL20/MIP3 alpha ELISA Kit

Catalog No: CKH171

Size: 1 x 96 tests

Introduction:

Chemokine (C-C motif) Ligand 20 (CCL20), also known as Macrophage Inflammatory Protein 3 alpha (MIP3A) is a small cytokine belonging to the C-C motif chemokine family. It is strongly chemotactic for lymphocytes and weakly attracts neutrophils. CCL20/MIP3A is implicated in the formation and function of mucosal lymphoid tissues via chemoattraction of lymphocytes and dendritic cells towards the epithelial cells surrounding these tissues. CCL20/MIP3A elicits its effects on its target cells by binding and activating the chemokine receptor CCR6. It plays a role in innate immunity by functioning as an antimicrobial peptide. Gene expression of CCL20/MIP3A can be induced by microbial factors such as lipopolysaccharide (LPS), and inflammatory cytokines such as Tumor Necrosis Factor (TNF) and Interferon gamma (IFNG), while Interleukin-10 (IL10) can down-regulate CCL20/MIP3A expression. CCL20/MIP3A is expressed in several tissues with highest expression observed in peripheral blood lymphocytes, lymph nodes, liver, appendix, and fetal lung and lower levels in thymus, testis, prostate and gut. It plays a critical role in the regulation of dendritic cell trafficking, and the recruitment and activation of T-cells.

The Human CCL20/MIP3A ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human CCL20/MIP3A in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for CCL20/MIP3A coated on a 96-well plate. Standards and samples are pipetted into the wells and CCL20/MIP3A present in a sample is bound to the wells by the immobilized antibody. The wells are washed and Biotinylated Anti-Human CCL20/MIP3A antibody is added. After washing away unbound Biotinylated antibody, HRP-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 CCL20/MIP3A bound.

Performance and Characteristics:

Sensitivity

The minimum detectable dose of CCL20/MIP3A is typically less than 1.5 pg/mL.

Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<12%



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Recovery

Recovery was determined by spiking various levels of Human CCL20/MIP3A into Human serum, plasma and cell culture media. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|--------------------|--------------------|-----------|
| Serum | 95.39 | 83-103 |
| Plasma | 93.88 | 82-102 |
| Cell culture media | 94.25 | 83-102 |

Linearity

| Sample Type | | Serum | Plasma | Cell culture media |
|-------------|-----------------------|--------|--------|--------------------|
| 1:2 | Average % of Expected | 93 | 92 | 94 |
| | Range (%) | 82-102 | 82-102 | 83-103 |
| 1:4 | Average % of Expected | 95 | 94 | 96 |
| | Range (%) | 94-103 | 83-102 | 84-103 |
| 1:8 | Average % of Expected | 94 | 96 | 95 |
| | Range (%) | 83-102 | 84-103 | 83-102 |

Reagents and materials supplied in the kit:

| Items | Quantity |
|--|----------|
| A. Microplate coated with Anti-Human CCL20/MIP3A | 96 wells |
| B. Wash Buffer Concentrate (20x) | 25 mL |
| C. Recombinant Human CCL20/MIP3A Standards | 2 vials |
| D. Assay Diluent A: Standard/Sample-Serum/Plasma * | 30 mL |
| E. Assay Diluent B (5x): Standard/Sample-Cell Culture Medium/Urine | 15 mL |
| F. Detection Antibody: Anti-Human CCL20/MIP3A | 2 vials |
| G. Streptavidin-HRP Concentrate (15,000x) | 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.



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.



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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/urine.

Assay Diluent B

Dilute 5-fold with deionized or distilled water.

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.

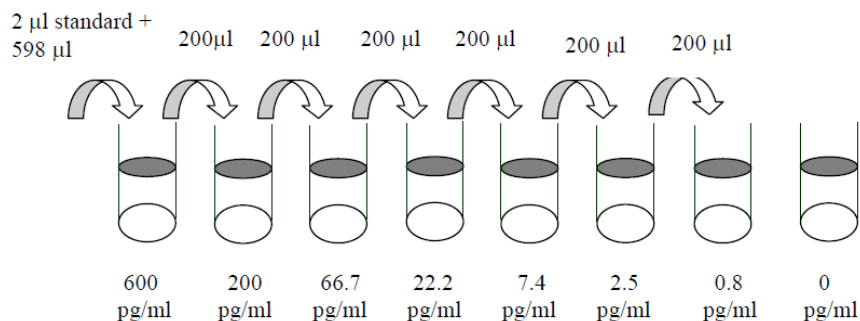
CCL20/MIP3A Standard

- Briefly spin the vial of Item C (Recombinant Human CCL20/MIP3A Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium/urine) to prepare a 180 ng/mL standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 2 µl standard from the vial of Item C, into a tube with 598 µl Assay Diluent A or 1x Assay Diluent B to prepare a 600 pg/mL stock standard solution.



- Pipet 400 μ l Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
- Mix each tube thoroughly before the next transfer. Gently vortex to mix.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL).

Figure 1



Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 μ l of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipet 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 80-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 15,000-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipet up and down to mix gently. Add 2 μ l of Streptavidin-HRP concentrate into a tube with 198 μ L 1x Assay Diluent B to prepare a 100-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly and then pipet 100 μ l of prepared 100-fold diluted solution into a tube with 15 mL 1x Assay Diluent B to prepare a final 15,000-fold diluted Streptavidin-HRP 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: CCL20/MIP3A Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C.
3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each).
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.
5. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each).
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.
7. Discard the solution and wash 5 times with 1x Wash Solution (300 µl each).
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.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.



Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.



2. Add 100 μ l standard or sample to each well.
Incubate 2.5 hours at room temperature or overnight at 2-4°C.



3. Add 100 μ l prepared biotin antibody to each well.
Incubate 1 hour at room temperature.



4. Add 100 μ l prepared Streptavidin solution.
Incubate 45 minutes at room temperature.



5. Add 100 μ l TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.



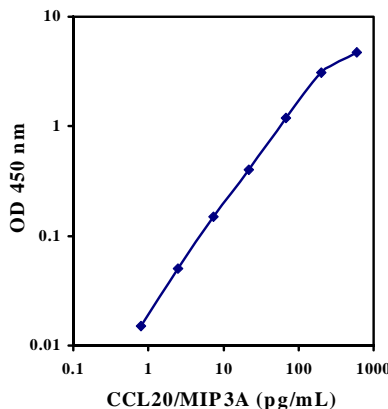
6. Add 50 μ l Stop Solution to each well. Read at 450 nm immediately.

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.

Typical Data:

Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



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. |

Disclaimer - This information is believed to be correct, but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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