CCL19/MIP-3 beta (Human)

ELISA KIT PROTOCOL

(Catalog No.: EK-045-68)

PHOENIX PHARMACEUTICALS, INC.

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CAUTION:

Investigational device. Limited by law to investigational use. For research use only. Not for use in diagnostic procedures.

INTRODUCTION

Macrophage Inflammatory Protein-3 beta (MIP-3β), also called CCL19, is a chemokine that is expressed in the thymus, lymph nodes and activated bone marrow stromal cells. It may play a role not only in inflamatory and immunological responses but also in normal lymphocyte recirculation and homing. May play an improtant role in trafficking of T-cells in thrymus, and T-cell and B-cell migration to secondary lymphoid organs. MIP-3β signals through the G protein-coupled receptor, CCR7, to regulate normal lymphocyte recirculation and homing of T and B cells to the lymph nodes. Human MIP-3β shows activity on mouse cells. Human CCL19 shows potent chemotactic activity for T-cells and B-cells but not for granulocytes an dmonocytes. Recombinant Human MIP-3 β is a non-glycosylated protein, consisting of 83 amino acids, with a molecular weight of 9.2 kDa.

Phoenix Pharmaceutical's Human CCL19/MIP-3 beta ELISA Kit is designed to measure the concentration of Human CCL19/MIP-3 beta from human serum/plasma, or cell culture supernatants.

PROTOCOL OVERVIEW

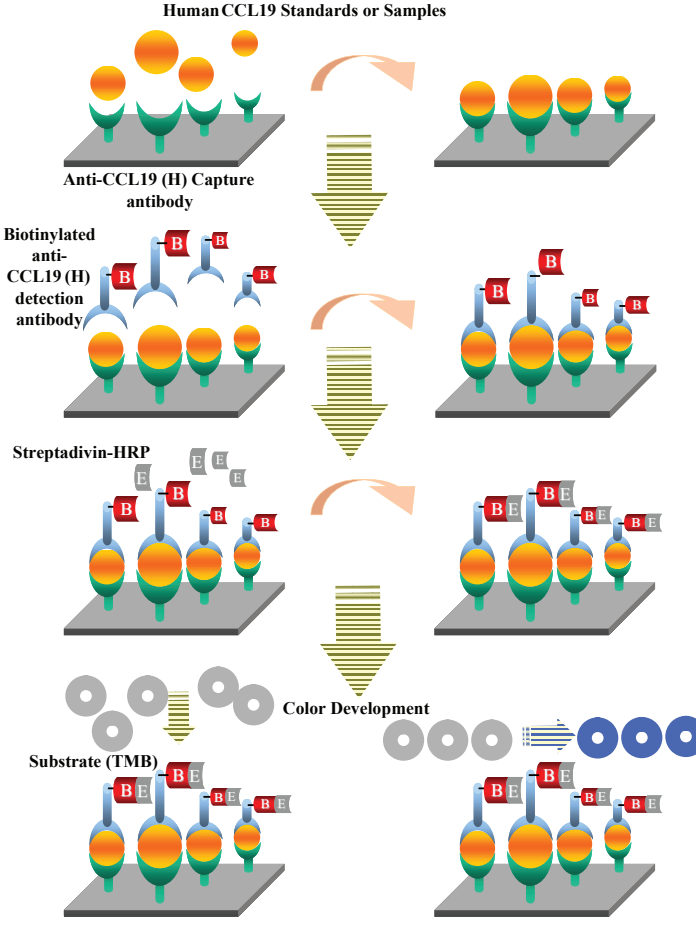
The immunoplate in this kit is precoated with anti-CCL19/MIP-3 beta Capture Antibody and the nonspecific binding sites are blocked. The Human CCL19/MIP-3 beta in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the Biotinylated anti-CCL19/MIP-3 beta Detection Antibody which can bind to the CCL19/MIP-3 beta trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the Substrate Solution (TMB) is added. The enzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the the color is directly proportional to the amount of CCL19/MIP-3 beta in the standard solutions or samples. A standard curve of CCL19/MIP-3 beta with known concentration can be established accordingly. The CCL19/MIP-3 beta with unknown concentration in samples can be determined by extrapolation to this standard curve.

ASSAY CONDITIONS

Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough the for the sensitivity of the kit to detect it.

Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

Assay Principle



LIST OF COMPONENTS

Store all components at 4°C. DO NOT FREEZE.

1. 20x Assay Buffer Concentrate *(50ml)*.....**Catalog No. EK-BUF**
2. 1x Diluent Buffer ..... **Catalog No. EK-DB-045-68**
3. 96 Well anti-CCL19/MIP-3 beta.....**Catalog No. EK-Plate-045-68**
   Capture Antibody-Coated Plate *(1 plate)*
4. Human CCL19/MIP-3 beta Standard.....**Catalog No. EK-S-045-68**
   (1000pg/vial)
5. Biotinylated anti-CCL19/MIP-3 beta.....**Catalog No. EK-D-045-68**
   Detection Antibody *(1 vial)*
6. Human CCL19/MIP-3 beta..... **Catalog No. EK-PC-045-68**
   Positive Control*(2 vials)*
7. Streptavidin-Horseradish..... **Catalog No. EK-SA-HRP**
   Peroxidase (SA-HRP) *(30µl)*
8. Substrate Solution (TMB) *(12ml)*.....**Catalog No. EK-SS**
9. Stop Solution 2N HCl *(15ml)* .....**Catalog No. EK-HCL**
10. Acetate Plate Sealer (APS) *(3 pieces)*.....**Catalog No. EK-APS**
11. Assay Diagram *(1 sheet)*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettor(s) and disposable pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution Reservoir *(recommended)*
- Microtiter plate washer *(recommended)*
- Orbital plate shaker capable of 300-500 rpm *(recommended)*
- Microtiter plate reader capable of absorbance measurement 450nm
- Well-closed containers (15ml tubes or more in capacity)
- Absorbent material for blotting

REAGENT PREPARATION

Note: *The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.*

1. **1x Assay Buffer:** Dilute the **20x** Assay Buffer Concentrate with 950ml of distilled water. This assay buffer will be used to wash the plate. If crystals are observed in the **20x** Assay Buffer warm the bottle in a 37°C water bath for approximately 30 minutes or until the crystals disappear. After preparation, store **1x** Assay Buffer at 4°C.
2. **Biotinylated anti-Human CCL19/MIP-3 beta Detection Antibody:** Rehydrate Biotinylated anti-Human CCL19/MIP-3 beta Detection Antibody with 100µl of **1x** Assay Buffer, vortex (centrifuge the tube to dislodge powder from the cap or walls). Dilute Biotinylated anti-Human CCL19/MIP-3 beta Detection Antibody to 1:90 and mix thoroughly before use.
3. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the SA-HRP vial (30µl) provided in this kit (3,000-5,000 rpm, 5 seconds) and dilute SA-HRP with **1x** Assay Buffer to 1:1000 before use. Vortex thoroughly.
4. **Human CCL19/MIP-3 beta Positive Control:** Rehydrate Human CCL19/MIP-3 beta Positive Control with 250µl of **1x** Assay Buffer (centrifuge the tube to dislodge powder from cap or walls). Vortex thoroughly.
5. **1x Diluent Buffer (Ready for use):** This buffer will be used to reconsititue all of the other components in this kit.
6. **Sample Preparation:** Dilute serum and/or plasma sample of 1:4, 1:8 or 1:16 in sample diluent buffer. It is recommended that each lab determine their appropriate dilution factor for their samples to obtain optimal results. Vortex thoroughly.

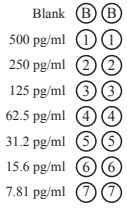
HUMAN CCL19/MIP-3 beta STANDARD PREPARATION

1. Rehydrate recombinant Human CCL19/MIP-3 beta Standard with 1ml **1x** Diluent Buffer. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. Vortex and centrifuge before use. The concentration of this stock solution is 1000pg/ml.

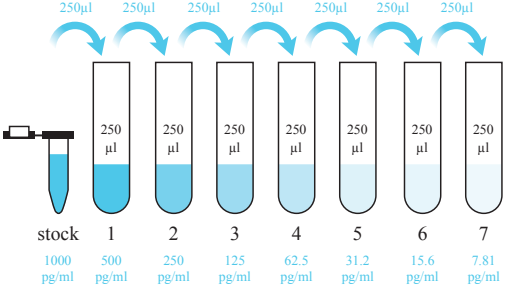
2. Prepare Standard solutions as follows:

Standard No.	Standard Protein Volume	Diluent buffer Volume	Concentration
Stock	Powder	1ml	1000pg/ml
#1	250µl of #Stock	250µl	500pg/ml
#2	250µl of #1	250µl	250pg/ml
#3	250µl of #2	250µl	125pg/ml
#4	250µl of #3	250µl	62.5ng/ml
#5	250µl of #4	250µl	31.25pg/ml
#6	250µl of #5	250µl	15.62pg/ml
#7	250µl of #6	250µl	7.81 pg/ml

Table of Standard Dilutions



Immunoplate loading map



Visual Guide of the Standard Dilutions

HUMAN CCL19/MIP-3 beta ELISA PROTOCOL

1. Thoroughly read this protocol before performing an assay. Allow all reagents to come to room temperature (20-23°C) prior to the start of the assay.
2. Remove Capture Antibody-Coated Plate from its zip-lock foil pouch. Remove unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 4°*C*.
3. Leave wells A-1 and A-2 empty as **Blank**.
4. Add 100µl of the prepared Human CCL19/MIP-3 beta Standard solutions from **#7** to **#1** (reverse order of serial dilution) in duplicate to each well.
5. Add 100µl of Human CCL19/MIP-3 beta Positive Control solution in duplicate.
6. Add 100µl diluted samples in duplicate into their designated wells.
7. Seal the immunoplate with Acetate Plate Sealer (APS). Incubate for 2 hours at room temperature (20-23°C) on a plate shaker (300-400 rpm).
8. Before washing the plate, remove the plate sealer carefuly. Completely discard the liquid from wells. Wash each well with 300-350µl assay buffer four times. At the end of each wash, discard the buffer, invert the plate, and tap on a clean absorbent towel.
9. Add 100µl of prepared Biotinylated anti-Human CCL19/MIP-3 beta Detection Antibody into each well. Reseal the immunoplate with plate sealer and incubate for 2 hour at room temperature (20-23°C) on a plate shaker (300-400rpm).
10. Wash 4 times with the **1x** Assay Buffer as described in step 8.
11. Add 100µl SA-HRP solution into each well. Reseal the immunoplate with plate sealer and incubate the plate for 20 minutes at room temperature (20-23°C) on plate shaker (300-400rpm).

13. Wash 4 times with the **1x** Assay Buffer as described in step 8.
14. Add 100µl Substrate Solution (TMB) provided in this kit into each well. Reseal the plate with plate sealer to protect from light and incubate the plate for 10-20 minutes at room temperature (20-23°C) on a plate shaker (300-400 rpm).
15. Add 100µl Stop Solution (2N Hydrochloric Acid) into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uniform, gently tap the plate to ensure thorough mixing. Proceed to the next step within 20 minutes.
16. Read Absorbance O.D. at 450nm using a Microtiter Plate Reader.

ADDITIONAL RECOMMENDED PROCEDURAL NOTES:

- Reagents of different lot numbers should not be mixed.
- Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
- Unused microplate strips should be placed in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- When handling the plate, avoid touching the bottom.
- Manual washing may cause high duplicate coefficient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
- If the room temperature is not within the suggested range (20-23°C), variations in results may occur.
- The same reservoir for the reagents may be reused if the reservoir is washed well with distilled water before each use.
- Each laboratory must determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve.
- High levels of interfering proteins may cause variations within the sample results. Therefore, it is imperative to select the appropriate sample preparation procedure to obtain the optimal results.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-500 rpm is recommended for all incubations.
- Modification of the existing protocol (i.e. standard dilutions, pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity and specificity of the test.

SUMMARY OF ASSAY PROTOCOL

Add 100µl/well of CCL19/MIP-3 beta standard, sample, or positive control **except the Blank wells**

▼

Incubate at room temperature (20-23°C) for 2 hours

▼

Wash immunoplate 4 times with 350µl/well of 1x assay buffer

▼

Add 100µl/well of Biotinylated anti-CCL19/MIP-3 beta Detection Antibody

▼

Incubate at room temperature (20-23°C) for 2 hours

▼

Wash immunoplate 4 times with 350µl/well of **1x** assay buffer

▼

Add 100µl/well of SA-HRP solution

▼

Incubate at room temperature (20-23°C) for 20 minutes

▼

Wash immunoplate 4 times with 350µl/well of **1x** assay buffer

▼

Add 100µl/well of Substrate Solution (TMB)

▼

Incubate at room temperature (20-23°C) for 20-30 minutes

▼

Terminate reaction with 100µl/well of 2N HCL

▼

Read absorbance O.D. at **450nm** and calculate results

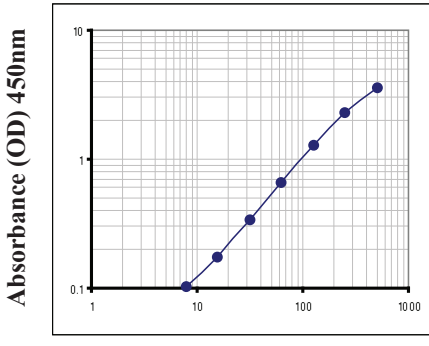
CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentration of Human CCL19/MIP-3 beta Standard and its corresponding O.D. reading is plotted on the log scale (X-axis) and the log scale (Y-axis), respectively. The standard curve shows a correlated relationship between Human CCL19/MIP-3 beta concentrations and the corresponding O.D. absorbance. As the standard concentration increases, the intensity of the yellow color, and in turn the O.D. absorbance, increases.

The concentration of Human CCL19/MIP-3 beta within a sample is determined by plotting the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the X-axis at a coordinate corresponding to the Human CCL19/MIP-3 beta concentration in the unknown sample.

Refer to QC Data sheet for acceptable values of the positive conrtol.

Human CCL19/MIP-3 beta Standard Curve



STORAGE

1. Store the kit at 4°C upon receipt. The kit should be equilibrated to room temperature (20-23°C) before assay.
2. Store **1x** Assay Buffer at 4°C.
3. Remove any unneeded strips from Human CCL19/MIP-3 beta antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human CCL19/MIP-3 beta Standard, Biotinylated anti-Human CCL19/MIP-3 beta Detection Antibody and SA-HRP at 4°C. Prepare only the required amount.

NOTE:

1. It is recommended that the solutions be used on the same day of rehydration.
2. Unextracted serum samples of normal sujects are to be diluted with 1x Diluent Buffer.
3. After adding Stop Solution, read the plate within 20 minutes.

REFERENCES

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