MIG/CXCL9 (Human)
ELISA Kit Protocol

(Cat. No.:EK-033-15)

PHOENIX PHARMACEUTICALS, INC.
330 Beach Road, Burlingame CA
Tel: 650-558-8898♦800-988-1205♦Fax: 650-558-1686
E-Mail: Info@PhoenixPeptide.com
www.PhoenixPeptide.com

TABLE OF CONTENT

INTRODUCTION AND PROTOCOL OVERVIEW

Both mRNA and protein expression of MIG/CXCL9 are present in the thryroid glands of patients who exhibit the on-set of Graves Disease. Furthermore, tonsil liquid taken from patients with streptococcal pharyngitis contains high amounts of the interferon (IFN)-dependent CXC chemokine known as monokine induced by IFN-gamma (MIG)/CXCL9 in the pressence of bacteria. The CXC chemokines MIG/CXCL9, IFN-inducible protein showed antibacterial activity agains S. pyrogenes, and inhibition of MIG/CXCL9 expression reduced the antibacterial activity at the surface of inflamed pharyngeal cells.

Phoenix Pharmaceutical's Human MIG/CXCL9 ELISA Kit is designed to measure the concentration of Human MIG/CXCL9 from human serum/plasma, or conditioned medium.

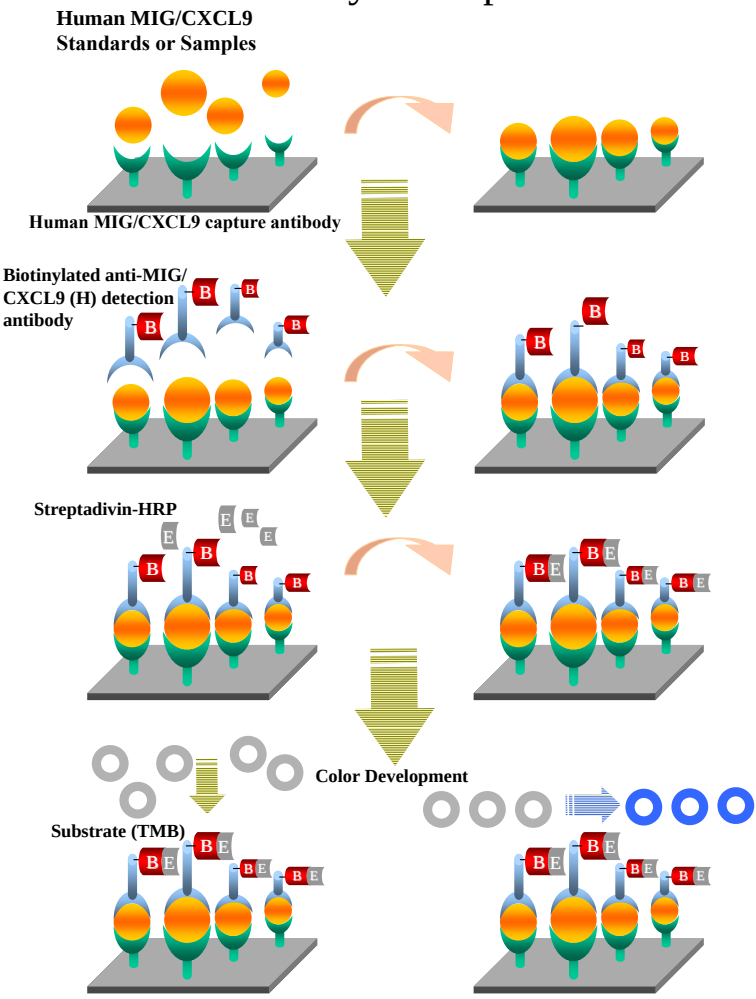
The immunoplate in this kit is precoated with Anti-Human MIG/CXCL9 Capture Antibody and the nonspecific binding sites are blocked. The Human MIG/CXCL9 in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the biotinylated anti-human MIG/CXCL9 Detection Antibody which can bind to the Human MIG/CXCL9 trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the substrate solution (TMB) is added. The exzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the the color is directly proportional to the amount of Human MIG/CXCL9 in the standard solutions or samples. A standard curve of Human MIG/CXCL9 with known concentration can be established accordingly. The Human MIG/CXCL9 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.

CAUTION: 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. 96 Well Anti-Human MIG/CXCL9.....**Catalog No. EK-Plate-033-15**
   Capture Antibody-Coated Plate *(1 plate)*

3. Human MIG/CXCL9 Standard.....**Catalog No. EK-S-033-15**
   (16ng/vial)

4. Biotinylated Anti-Human MIG/CXCL9.. **Catalog No. EK-D-033-15**
   Detection Antibody *(1 vial)*

5. Human MIG/CXCL9 Positive Control.. **Catalog No. EK-PC-033-15**
   (2 vials)

6. Streptavidin-horseradish peroxidase..... **Catalog No. EK-SA-HRP**
   (SA-HRP) *(30µl)*

7. Sustrate Solution (TMB) *(12ml)*..... **Catalog No. EK-TMB**

8. Stop Solution 2N HCl *(15ml)*..... **Catalog No. EK-HCL**

9. Acetate plate sealer (APS) *(3 pieces)*..... **Catalog No. EK-APS**

10. Assay Diagram *(1 sheet)*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettor(s) and disposable pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution Resevoir *(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

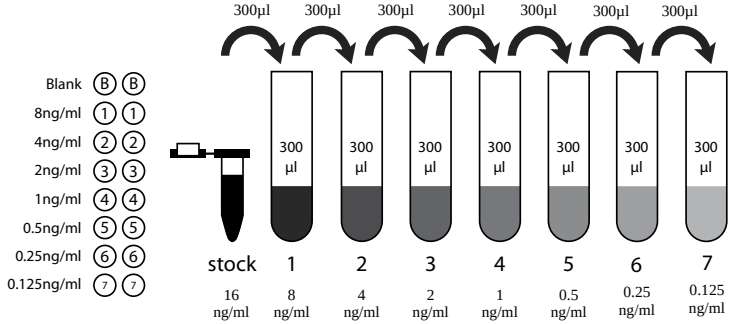
Note: *The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recom nended that the solutions be used as soon as possible after rehydra tion.*

1. **1x Asssay buffer:** Dilute the **20x** assay buffer concentrate with 950ml of dustilled water. This assay buffer will be used to wash the plate and reconstitute all of the other components in this kit. 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 cystals disappear. After preparation, store **1x** assay buffer at 4°C.
2. **Biotinylated anti-Human MIG/CXCL9 Detection Antibody:** Rehydrate biotinylated anti-Human MIG/CXCL9 Detection Antibody with 100µl of **1x** assay buffer, vortex (centrifuge the tube to dislodge powder from the cap or walls). Dilute biotinylated anti-Human MIG/CXCL9 Detection Antibody to 1:200 and mix thoroughly before use.
3. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the HRP vial (30µl) provided in this kit (3,00-5,000 rpm, 5 seconds) and dilute HRP with **1x** assay buffer to 1:2000 before use. Vortex toroughly.
4. **Human MIG/CXCX9 Positive Control:** Rehydrate Human MIG/CXCL9 Human Positive Control with 250µl of **1x** assay buffer (centrifuge the tube to dislodge powder from cap or walls). Vortex toroughly.

HUMAN MIG/CXCL9 STANDARD PREPARATION

1. Rehydrate recombinant Human MIG/CXCL9 standard with 1 ml **1x** asssy buffer, vortex. Allow the solution to sit at least 10 min utes at room temperature (20-23°C) to completely dissolve in solu tion. Vortex and centrifuge before use. The concentration of this stock solution is 16ng/ml.
2. Prepare Human MIG/CXCL9 standard solutions as follows:

Standard No.	Std. volume	Assay Buffer	Concnetrations
Stock	Powder	1000µl	16ng/ml
#1	300µl of Stock	300µl	8ng/ml
#2	300µl of #1	300µl	4ng/ml
#3	300µl of #2	300µl	2ng/ml
#4	300µl of #3	300µl	1ng/ml
#5	300µl of #4	200µl	0.5ng/ml
#6	300µl of #5	200µl	0.25ng/ml
#7	300µl of #6	300µl	0.125ng/ml



HUMAN MIG/CXCL9 ELISA PROTOCOL

1. Thorouhgly read this protocol before performing an assay. Al low 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. Wash each well with 300µl of **1x** assay buffer. Allow it to sit for at least five minutes. Discorad the buffer, invert and blot dry plate. Do not let wells dry before proceeding to the next step.
4. Leave wells A-1 and A-2 empty as **Blank**.

5. Add 100µl of the prepared Human MIG/CXCL9 Standard solu tions from **#7** to **#1** (reverse order of serial dilution) in duplicate to each well.
6. Add 100µl of Human MIG/CXCL9 positive control solution in duplicate.
7. Add 100µl diluted samples in duplicate into their designated wells.
8. 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).
9. 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 the wash, discard the buffer, invert the plate, and tap on a clean absorbent towel.
10 Add 100µl biotinylated anti-Human MIG/CXCL9 Detection An tibody into each well. Reseal the immunoplate with plate sealer and incubate for 2 hours at room temperature (20-23°C) on a plate shaker (300-400rpm).
11. Wash 4 times with the **1x** assay buffer as described in step 9.
12. Add 100µl SA-HRP solution into each well. Reseal the im munoplate with plate sealer and incubate the plate for 30 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 9.
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 20-30 minutes at room tempera ture (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 shoud 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 dessicant 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 appropeate 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 otimal 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 Human MIG/CXCL9 Standard, Positive Control, or diluted Samples

↓

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

↓

Wash immunoplate 4 times with 300-350µl/well of **1x** assay buffer

↓

Add 100µl/well Biotinylated anti-Human MIG/CXCL9 Detection Antibody

↓

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

↓

Wash immunoplate 4 times with 300-350µl/well of **1x** assay buffer

↓

Add 100µl/well of SA-HRP solution

↓

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

↓

Wash immunoplate 4 times with 300-350µl/well

↓

Add 100µl/well of substrate solution (TMB)

↓

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

↓

Terminate the reaction with 100µl/well of Stop Solution

↓

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

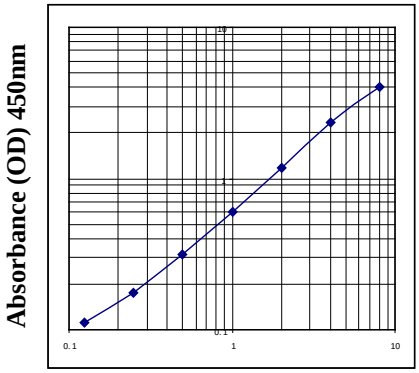
CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentration of Human MIG/CXCL9 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 MIG/CXCL9 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 MIG/CXCL9 in 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 MIG/CXCL9 concentration in the unknown sample.

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

Human MIG/CXCL9 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 un-needed strips from Human MIG/CXCL9 Antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human MIG/CXCL9 Standard, Biotinylated anti-Human MIG/CXCL9 Detection Antibody and SA-HRP at 4°C. Prepare only the required amount.

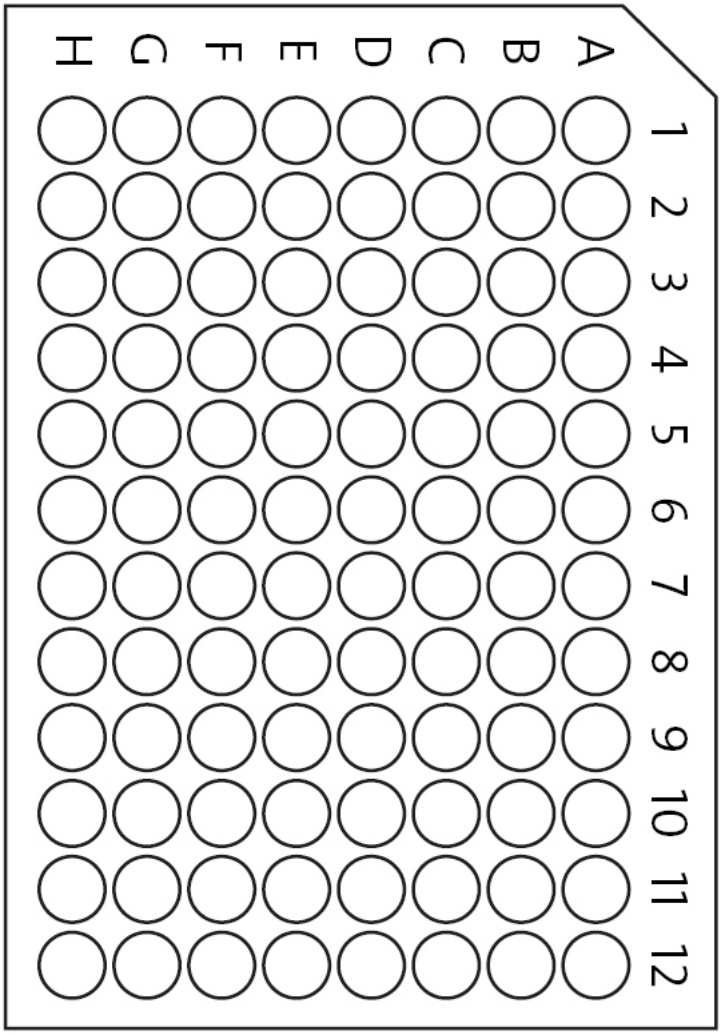
NOTE:

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

REFERENCES

1. Linge HM, Sastalla I, Nitsche-Scmitz DP, Egesten A., Frick IM. Protein FOG is a moderate inducer of MIG/CXCL9, and group G streptococci are more tlerant than group A sterptococci to this chemokine's antibacterial effect. Department of Clinical Sciences Lund, Division of Medicine, Lund University, BMC, B14, SE-221 84 Lund, Sweden.
2. Egesten A, Eliasson M, Johansson HM, Olin AI, Morgelin M, Mueller A, Pease JF, Frick IM, Bjorck L. The CXC chemokine MIG/CXCL9 is important in innate immunity against Streptococcus pyogenes. Section of Respiratory Medicine, Department of Clinical Sciences, Lund University, Lund, Sweden.
3. Romagnani P, Rotondi M, Lazzeri E, Lasagni L, Francalanci M, Buonamano A, Milani S, Vitti P, Chiovato L, Tonacchera M, Bellastella A, Serio M. Expression of IP-10/CXCL10 and MIG/CXCL9 in the thyroid and increased levels of IP-10/CXCL10 in the serum of patients with recent-onset Graves' disease. Department of Clinical Pathophysiology, University of Florence, Italy.

ASSAY DIAGRAM



NOTES