CHEMICON®
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ChemiKine™ Human Macrophage Inhibitory Factor (MIF) EIA Kit

Cat. No. CYT1001

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Application

ChemiKine™ Human MIF EIA Kit is designed to measure MIF in various samples such as serum, body fluid, buffered solutions and in cell culture medium. The assay will recognize both natural and recombinant Human MIF. There are enough reagents included in this kit for one 96-well immunoassay plate. We recommend running duplicate wells for the standards and unknowns.

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Test Principle

With the ***ChemiKine***™ assay system, pre-coated mouse monoclonal antibodies generated against human MIF are used to capture human MIF in a sample. Simultaneously, HRP-labeled MIF specific goat polyclonal antibodies bind to MIF in the sample. MIF in the sample is detected by the addition of Luminescent HRP Substrate. The standard curve demonstrates a direct relationship between sample luminescence (CPS) and MIF concentration: i.e., the higher the CPS the higher the MIF concentration in the sample.

Kit Components

1. 20X EIA Assay Buffer: - (Part No. 2004914). One vial (5ml) of 20X Assay Buffer.
2. 20X TBS - (Part No. 2004913). One vial (50 ml) of 20X TBS.
3. 20% Tween-20™ - (Part No. 2004915). One vial (3 ml) of 20% Tween-20™.
4. Anti-human MIF, HRP Conjugate (400X) - (Part No. 2004916). One vial (30 µl) of Anti-MIF-HRP conjugated antibody.
5. MIF Standard - (Part No. 2004918). One vial of recombinant human MIF, lyophilized.
6. LumiGlo® Chemiluminescent Substrate A - (Part No. 2004919). One bottle (10 mL) of HRP Chemiluminescent Substrate A.
7. LumiGlo® Chemiluminescent Substrate B - (Part No. 2005070). One bottle (10 mL) of HRP Chemiluminescent Substrate B.
8. Immunoplate Pre-Coated with Mouse Anti-MIF Monoclonal Antibody - (Part No. 2004917). One pre-coated 96-well plate
9. Plate Covers - Two Plate Covers.

Materials Not Supplied

1. Multi-channel or repeating pipettes
2. Pipettors & tips capable of accurately measuring 5 to 1000 µL
3. Graduated serological pipets, 25 mL and/or 10 mL
4. 96-well luminescent microtiter plate reader
5. 96-well plate washer with gravity feed (optional)
6. Graphing software for plotting data
7. 1.5 ml tubes
8. Mechanical vortex
9. Two 0.5-liter containers
10. 15 mL and 50 mL centrifuge tubes
11. Distilled or deionized water.

Precautions

The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in the sub-optimal performance of the kit and the failure to produce accurate data.

Storage

Prior to use maintain the kit at 2°-8°C until expiration date.

1. MIF Standard - After reconstitution maintain the Stock Standard in undiluted aliquots for one month at 2°-8°C or up to six months at -20°C. If standard is stored at -20°C, aliquot to avoid repeated freeze-thaw cycles.
2. Wash Buffer – After dilution maintain the Wash Solution at 2°-8°C for up to six months.
3. Tween-20™ - Store at room temperature upon receipt of kit.
4. EIA Assay Buffer – After dilution maintain the EIA Assay Buffer at 2°-8°C for up to one month
5. Anti-human MIF, HRP Conjugate, TBS, LumiGlo® Substrates, and Immunoplate - Stable until expiration date if maintained at 2°-8°C.

Preparation of Reagents

1. Wash Buffer

Warm the 20X TBS concentrate to room temperature and mix to ensure that any precipitated salts have re-dissolved. For 500 mL of Wash Buffer, combine 25 mL of 20X TBS, 1.25 mL of 20% Tween-20, and 473.75 mL distilled or deionized water. Stir to homogeneity.

2. TBS

Warm the 20X TBS concentrate to room temperature and mix to ensure that any precipitated salts have re-dissolved. Dilute 1 volume of the 20X TBS concentrate with 19 volumes of distilled or deionized water. Stir to homogeneity.

3. EIA Assay Buffer

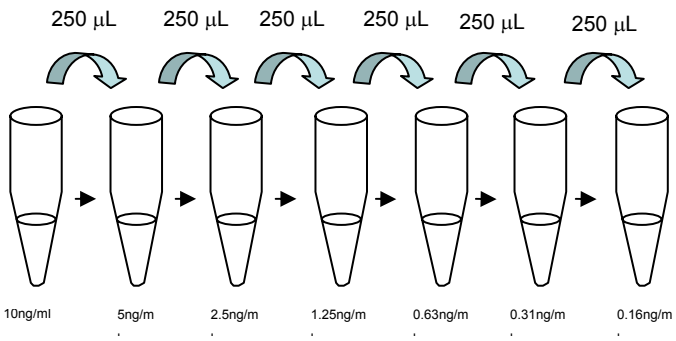
Warm the 20X concentrate to room temperature and mix to ensure that any precipitated salts have re-dissolved. Dilute 1 volume of the 20X EIA Assay Buffer concentrate with 19 volumes of distilled or deionized water. Stir to homogeneity.

4. Human MIF Standard

a) Label 7 test tubes 0.15625, 0.3125, 0.625, 1.25, 2.5, 5 and 10 ng/mL. Add 250 µL of 1X EIA Assay Buffer to each of the tubes.

b) Reconstitute the MIF Standard with 1.25 mL of 1X EIA Assay Buffer. This is the stock standard which has a concentration of 100 ng/mL. For the standard curve, dilute the stock MIF to 20 ng/mL by adding 60 µL to 240 µL of 1X EIA Assay Buffer and vortex. Add 250 µL of this diluted material to the tube labeled 10 ng/mL.

c) The remaining standards are then prepared by performing a 1:2 dilution of the preceding standard. For example, to make the 5 ng/mL Standard add 250 µL of the 10 ng/mL standard to the 5 ng/mL tube and vortex, to make the 2.5 ng/mL Standard, add 250 µL of the 5 ng/mL Standard to the 2.5 ng/mL tube and vortex, and so on. Use 100 µL of 1X EIA Assay Buffer for a zero MIF condition.



5. Anti-human MIF, HRP Conjugate

On the day of use dilute 2.5 µL of MIF-HRP Antibody Conjugate Concentrate with 1 mL of EIA Assay Buffer for each 8-well strip used in assay.

6. LumiGLO® HRP Chemiluminescent Substrate

Mix Substrate A and Substrate B in equal volumes. For each 8-well strip used, dilute 375 µL of Substrate A with 375 µL of Substrate B and mix well. Once combined, the substrate solution is stable for 1 hour at room temperature and up to 24 hours when stored at 4°C.

7. Immunoplate.

Provided 'ready to use'.

Assay Instructions

The peroxidase reaction is temperature sensitive, therefore *these reagents must be at room temperature prior to use*. Gently mix all reagents.

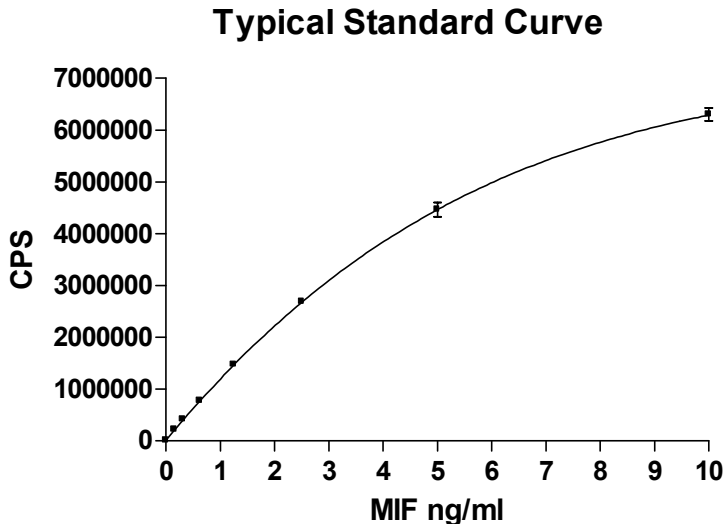
1. Determine the number of 8-well strips needed for the assay. Insert these into the frame for current use. (Re-bag the extra strips and frame. Return unused strips to refrigerator for future use.)
2. Dispense 100 µL of Standard control or sample per well. Cover wells with plate sealer and incubate on a plate shaker at room temperature for 2 hours. It is recommended that all standards and samples be run in duplicate.
   Note: *A standard curve must be run at each setting.*
3. Thoroughly aspirate or decant solution from wells and discard the liquid. Fill wells to top with 300 µL of diluted Wash Buffer. Decant or aspirate thoroughly. Repeat this wash step five times. Invert wells and allow to drain briefly on dry, absorbent paper.

4. Dispense 100 µL of the diluted Anti-human MIF, HRP Conjugate to each well. Cover wells with plate sealer and incubate on the plate shaker at room temperature for an hour.
5. Aspirate the wells and wash as in step 3 above.
6. Wash twice more using 1X TBS. Invert wells and allow to drain briefly on dry, absorbent paper.
7. Dispense 75 µL of the combined Luminescent substrates A and B.
8. Read the plate in a luminometer with 1 second integration time per well between 5 - 45 minutes after addition of substrate.

Calculation of Results

1. Calculate the mean luminescence for each set of standard wells.
2. A standard curve is generated by plotting the mean luminescence (y axis) against ng/mL standard (x axis).
3. Read the MIF concentration for unknown samples from the standard curve plotted in step 2. For best results, a 4-parameter logistic curve-fit (sigmoidal dose-response with variable slope) is recommended.

Sample Curve



Technical Hints

- When not in use, kit components should be stored refrigerated or frozen as described in *Storage* and *Kit Components* sections of this insert. All reagents should be warmed to room temperature before use.
- Do not use reagents beyond the expiration date of the kit.
- Do not mix or interchange different reagent lots from various kit lots.
- Cover or cap all reagents when not in use.
- Samples should be stored frozen if not analyzed shortly after collection.
- Avoid multiple freeze-thaw cycles. Thaw completely and mix well prior to analysis.
- When possible avoid use of hemolysates. If large amounts of particulate matter are present, results may indicate higher than accurate concentrations.
- Avoid contamination of sodium azide to the samples and reagents in the assay, as sodium azide strongly inhibits peroxidase activity.
- Samples that are outside the predictive range of the standard curve should be diluted with EIA Assay Buffer and re-tested.
- Samples should be assayed in duplicate.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation for all wells.
- Microplate should be allowed to come to room temperature before opening the foil bag. Once the desired number of strips has been removed, immediately reseal the back to maintain plate integrity. Store these in the refrigerator for future use.
- Take care not to scratch the inside of the well.
- Use only coated well from the same reagent batch for each assay.
- When washing and tapping the plate, never insert absorbent paper directly into the wells.
- Avoid exposing all reagents to direct sun light.
- All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.
- Please follow the manufacturer's instructions relating to the safe handling and use of all materials and equipment.

Analytical Sensitivity and Detection Limits

Sensitivity:	5 pg/mL
Range of Detection:	5 pg/mL to 10 ng/mL
Species Reactivity:	Human. Shows no cross reactivity with rat or mouse MIF.

Warranty

These products are warranted to perform as described in their labeling and in CHEMICON® literature when used in accordance with their instructions. THERE ARE NO WARRANTIES, WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON® DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CHEMICON®*'s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CHEMICON®, to repair or replace the products. In no event shall CHEMICON® be liable for any proximate, incidental or consequential damages in connection with the products.

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