HUMAN MEGAKARYOCYTE-POTENTIATING FACTOR (MPF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN MPF CONCENTRATIONS IN SERUM AND EDTA PLASMA

MPF IS DETECTABLE IN SALIVA. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN MPF ELISA
Catalog No.	SK00722-06
Lot No.	
Formulation	96 T
Standard range	0.25-16 ng/ml
Sensitivity	80 pg/ml
Sample Volume	100 µl
Sample Type	Serum, EDTA plasma
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Specificity	Human MPF
Calibration	Human MPF (HEK293 derived) recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human MEGAKARYOCYTE-POTENTIATING FACTOR (MPF) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human MPF from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human MPF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural MPF samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human MPF. The capture antibody can bind to the human MPF in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human MPF is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human MPF bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
MPF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against MPF.	**722-06-01**	**1 plate**
MPF Standard – refer to lot specific of recombinant human MPF in a buffered protein base with preservative; lyophilized.	**722-06-02**	**1 vial**
Detection Antibody Concentrate refer to lot specific, 10-fold concentrate of biotinylated antibody against MPF with preservative; lyophilized.	**722-06-03**	**1 vial**
Positive Control – one vial of recombinant human MPF; lyophilized.	**722-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 30 mL of buffered protein based solution with preservative.	**DB11**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB103**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB68C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 10 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C.
For Longer storage for Dilution Buffer (**DB11**) and Antibody Diluent Solution **(DB103**), store at -20°C.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C or -70°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C or 70°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Aliquot and store samples at -20°C ~ -70°C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order code: 00740-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum or Plasma Samples may need 4~16 fold of dilution. A 4-fold dilution is 60 µl of samples + 180 µl of dilution buffer. A 8-fold dilution is 30 µl of samples + 210 µl of dilution buffer. A 16-fold dilution is 15 µl of samples + 225 µl of dilution buffer.
Optimal dilutions should be determined by each laboratory for each application. A pretest is suggested to determine the dilution factor.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

MPF Standard - Reconstitute the MPF standard with refer to lot specific of Dilution Buffer (**DB11**). Pipette 250 µl of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The **16 ng/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/ml).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Refer to lot specific	
# 1	Refer to lot specific	Refer to lot specific	16 ng/ml
# 2	250 µl of 1	250 µl	8 ng/ml
# 3	250 µl of 2	250 µl	4 ng/ml
# 4	250 µl of 3	250 µl	2 ng/ml
# 5	250 µl of 4	250 µl	1 ng/ml
# 6	250 µl of 5	250 µl	0.5 ng/ml
# 7	250 µl of 6	250 µl	0.25 ng/ml

Positive Control - Reconstitute the Positive Control with refer to lot specific of Dilution Buffer (**DB11**).

Detection Antibody Concentrate - Reconstitute each Detection Antibody Concentrate vial with refer to lot specific of **Antibody Diluent Solution (DB103)** to produce a 10-fold concentrated stock solution. Pipette 9.45 ml of **Antibody Diluent Solution (DB103)** into a 15 ml centrifuge tube and transfer

1.05 ml of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB68C)** into a 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot specific on micro-plate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the MPF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	Refer to lot specific
0.25	0.063
0.5	0.133
1	0.263
2	0.577
4	1.146
8	1.897
16	2.767

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human MPF (HEK293)	100
Human Mesothelin (HEK293)	0
Human Mesothelin Fc (HEK293)	0
Human Periostin	0
Human SPARC	0
Human BD1	0

The recombinant human MPF derived from E. Coli may not be detected by this ELISA Kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate refer to lot specific on plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.