Tribioscience

Human MIP-1α ELISA Catalog Number: TBS3234

For the quantitation of human MIP-1α concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

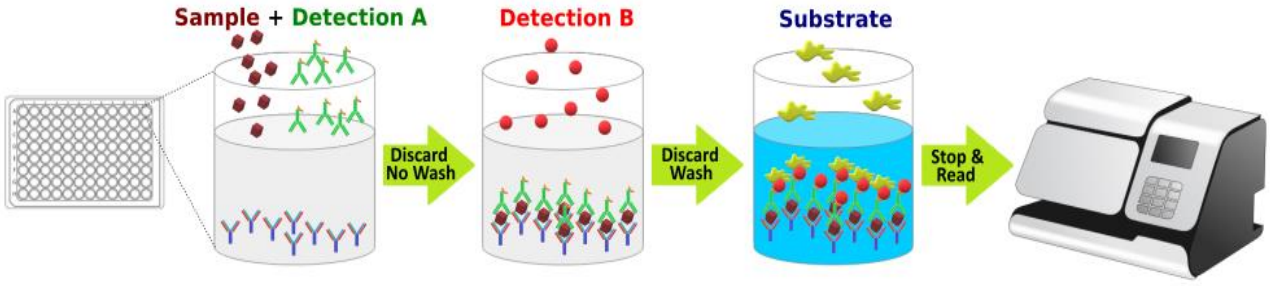
Macrophage Inflammatory Protein-1α (MIP-1α) is a member of the chemokine b or CC subfamily. Chemokines have been shown to have chemotactic activity and play critical roles in immunomodulation and processes of inflammation. The human MIP-1α cDNA encodes a signal peptide with 22 aa residues that is cleaved to produce the secreted mature protein. MIP-1α has been identified as a stem cell inhibitor (SCI) that inhibits the proliferation of hematopoietic progenitor cells in vitro and in vivo. In addition, MIP-1α is also recognized as a major HIV suppressor produced by CD8+ T cells.

The Fast Human MIP-1α ELISA is a solid phase ELISA designed to measure human MIP-1α levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, with no need for 4-5 hours (Fig. 1). The detection range is from 7 to 500 pg/mL. The levels of human MIP-1α samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human MIP-1α protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for human MIP-1α was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 45min. Then, aspirate each well, no wash, directly add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of MIP-1α bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1: Assay Principle:



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human MIP-1α Microplate	TBS3234A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human MIP-1α.	The unused wells can be stored the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human MIP-1α Standard	TBS3234B	60 ul of Recombinant human MIP-1α protein (10ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3234C	2.1 ml of human MIP-1α antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3234D	12 ml of Streptavidin-HRP	
Assay Diluent	TBS3234E	12 ml of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

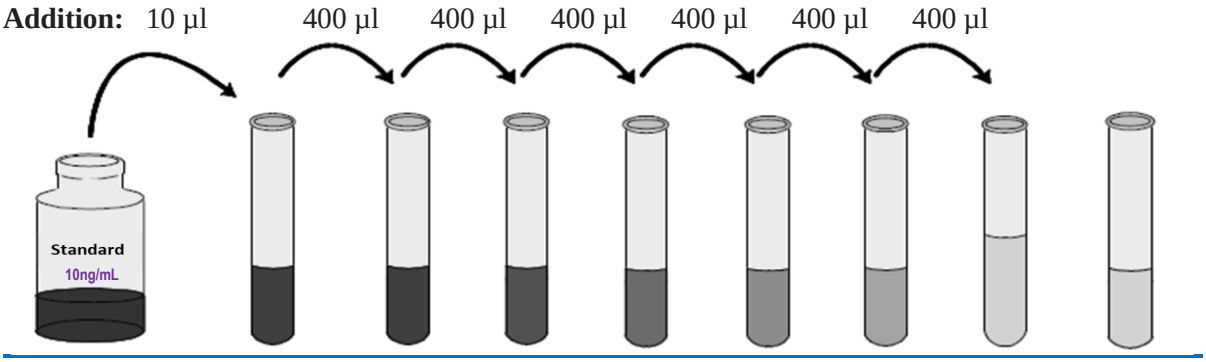
Bring all reagents to room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Human MIP-1α Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 475 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as diagram below (Fig. 2).**
2. Add 25 µL of the Human MIP-1α Standard stock solution (10ng/mL) by dilution of 20 times to tube #1 (500pg/mL) and mix.
3. Make 2.5x serial dilutions of the standard using the 500pg/mL standard solution in tube#1 from tube #2 through #7 with sequential transfer of 400 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 500; 250; 125; 62.5; 31.3; 15.63 and 7.813 pg/mL. Tube# 8 is Standard 0.

Fig. 2 Diagram for human MIP-1α standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	475	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol.(µL)	25	250	250	250	250	250	250	0
Final Conc (pg/ml)	500	250	125	62.5	31.3	15.63	7.813	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour.**
3. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 30min.**
5. Aspirate each well, and wash for 3 times by filling each well with 200 µL Wash Buffer (*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.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) 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 human MIP-1α 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.

TYPICAL DATA

This standard curve(R^2=0.9985) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human MIP-1α is typically 5 pg/ml.
The Intra-assay CV is 4.9%, the Inter-assay CV is 8.1%.

SPECIFICITY

This assay recognizes natural and recombinant human MIP-1α.

RELATIVE PRODUCTS

Human IL-α ELISA (TBS3202)
Human IL-1IL-1β ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human TNF-α ELISA (TBS3235)

For research use only.

Fig.3 Fast Human MIP-1α Standard