Human MIP3α ELISA kit
(DDXK-E-MIP3α)

Intended use
The human MIP3α ELISA kit is for quantitative determination of MIP3α concentrations in human cell culture supernatants and biological fluids (plasma, serum). This kit is for research purpose only.

Background
Macrophage inflammatory protein (MIP3α)/ CCL20 is a CC-type chemokine mapped to chromosome 2 in humans. Langerhans cells (LCs) represent a unique population of DCs colonizing epithelium. MIP3α plays a central role in LC precursor recruitment into the epithelium during inflammation. **(a)** Among DC populations, MIP3α is the most potent chemokine inducing the selective migration of *in vitro*-generated $CD34^+$ hematopoietic progenitor cell-derived LC precursors and skin LCs, in accordance with the restricted MIP3α receptor (CC chemokine receptor 6) expression by these cells. **(b)** MIP3α is mainly produced by epithelial cells, and the migration of LC precursors induced by the supernatant of activated skin keratinocytes is blocked by an antibody against MIP3α. **(c)** *In vivo*, MIP3α is selectively produced at sites of inflammation as illustrated in tonsils and lesional psoriatic skin where MIP3α up regulation is associated with an increase in LC turnover. **(d)** The secretion of MIP3α is strongly up regulated by cells of epithelial origin after inflammatory stimuli (interleukin 1β plus TNFα) or T cell signals (*Homey B. et al, 2000, J.Immunol. 164:6621-32).* Monoclonal antibodies anti-MIP3α were obtained after mice immunization with MIP3α-transfected eukaryotic cells. These clones were used to set up a human MIP3α specific Elisa, which also detects macaque MIP3α. No cross-reactivity with MIP1α, MIP1β, MIP1δ, MIP3β, eotaxin, 6Ckine, Rantes, MCP-1, TARC, MDC, TECK, SDF-1 and IP-10 was detected *(Dieu-Nosjean et al, J;Exp.med, 2000 192: 705-18).*

Kit contents
Capture Antibody: 0.5mg/mL of mouse anti-MIP3α monoclonal antibody (#DDX0430, clone 319F6).
Detection Antibody: 0.5mg/mL of HRPO-conjugated anti-MIP3α mouse monoclonal antibody (#DDX0420, clone 206D9).
Standard: Each vial contains 1µg/mL of recombinant human MIP3α produced, purified and concentrated from eukaryotic cells. A 7-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 20 ng /mL is recommended.

Storage
All the reagents should be aliquoted before storage. Minimize repeated freeze and thaw. Refer to expiration date on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot number. Capture and detection antibodies storage: -20°C

Materials and reagents required but not provided
96well-plate Nunc Maxi Sorp
50mM Carbonate buffer pH9.6
PBS-1% BSA-0.05% Tween20
TMB super sensitive HRP (TMBS100-0500, TEBU-BIO)
Multichannel pipettes and pipette tips
A standard microplate reader (620nm)

Sensitivity

The minimum detectable dose of human MIP3α was determined to be approximately 0.2ng/ml.

Principle of the assay

The human MIP3α ELISA kit is for the quantitative determination of human MIP3α in human cell culture supernatants, plasma, serum, and various biological fluids. This ELISA kit contains the specific components required for the development of human MIP3α sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on 3X96-well plates.

The DDX MIP3α ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). The capture monoclonal antibody specific for MIP3α is coated on a 96-well plate. Standards and samples are added to the wells, and any MIP3α present binds to the immobilized antibody. The wells are washed and a HRPO-conjugated anti-MIP3α monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of MIP3α present in the sample, TMB substrate solution is loaded and absorbance is measured at 620 nm.

Plate Preparation

Pre-warm all the reagents to room temperature prior to setting up the assay

1. Dilute the capture antibody to 2.5µg/ml in 50mM carbonate buffer pH9.6. Immediately coat a 96-well microplate with 120µL per well of the diluted capture antibody. Seal the plate and incubate overnight at room temperature (RT).
2. Remove coating solution, and wash with at least 200µl of PBS-0.05% Tween20, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.

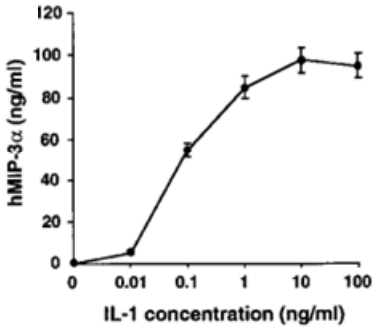
   The plates are now ready for sample addition.

Assay Procedure

1. Add 100µL/well of sample or standards, diluted in PBS-1% BSA-0.05% Tween20. Seal the plate and incubate 1h30 at 37°C.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100µL/well of the HRPO-conjugated detection antibody, diluted in PBS-1% BSA-0.05% Tween20 to 3µg/ml. Seal the plate and incubate 1h30 at 37°C.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100µL/well of TMB. Incubate for 5-10 minutes at RT.
6. Determine the optical density of each well, using a microplate reader set to 620 nm.

Standard curve

Each laboratory should establish its own standard curve. Here is an example of MIP3α dosage using DDX-K-E-MIP3α.



Elisa analysis of MIP-3α secretion by CHA cell line cultured in the presence of increasing doses of IL1-β

Dieu-Nosjean et al, 2000

Troubleshootings

To obtain good and reproducible results, usage of sterile reagents and clean materials is strongly recommended. All basic reagents such as washing and dilution buffers, water, must be devoid of contamination.

To ensure pH stability, incubation at 37°C should be performed in a humidified atmosphere of 5% CO_2

Problems	Possible Sources	Solutions
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Inappropriate storage	Aliquot standard and store at -70°C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature or timing	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples