Human MIP-1α Instant ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human MIP-1α

Catalog Number BMS2029INST 128 Tests

Pub. No. MAN0016488 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human MIP-1α Instant ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of MIP-1α levels in cell culture supernatants, serum, and plasma.

Summary

Chemokines are cytokines that induce chemotaxis of inflammatory cells. They are able to induce leukocyte chemotaxis and adhesion to endothelial cells.

Macrophage inflammatory protein 1α (MIP-1α) and 1β (MIP-1β) belong to the family of cysteine-cysteine (cc) chemokines, RANTES being another prominent member thereof. The chemokines self-associate to form high molecular mass aggregates while the monomers are low mass polypeptides. Both MIP-1α and MIP-1β are not only chemoattractants but also coactivators of macrophages acting in concert with IFN-γ as type 1 cytokines.

MIP-1α and MIP-1κ are distinct but highly homologous chemokines produced by a variety of host cells in response to various external stimuli and share affinity for their receptor CCR5. The roles of MIP-1α and MIP-1β have been elucidated in response to their affects on cellular and humoral immune response.

MIP-1α was shown to stimulate strong antigen specific responses, while MIP-1β promotes antibody responses.

Both macrophage inflammatory proteins are however strictly associated with type 1 immune response.

For literature update refer to our website.

Principles of the test

An anti-human MIP-1α polyclonal coating antibody is adsorbed onto microwells. Human MIP-1α present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-human MIP-1α antibody binds to human MIP-1α captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human MIP-1α.

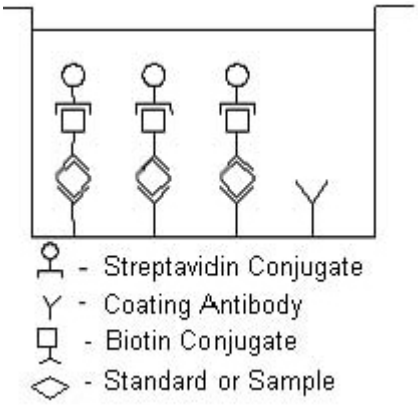

Fig. 1 Second incubation.

Following incubation unbound biotin conjugated anti human MIP-1α and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

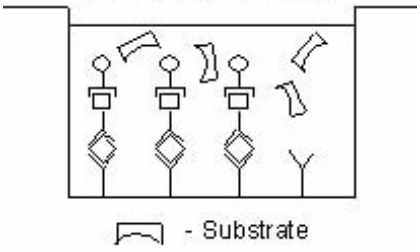

Fig. 2 Third incubation.

A colored product is formed in proportion to the amount of soluble human MIP-1α present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human MIP-1α standard dilutions and human MIP-1α sample concentration determined.

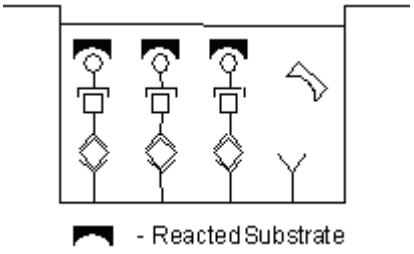

Fig. 3 Stop reaction.

Reagents provided

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with Polyclonal Antibody to MIP-1α, Biotin-Conjugate (anti-MIP-1α polyclonal antibody), Streptavidin-HRP, and sample diluent, lyophilized

2 aluminum pouches with a human MIP-1α standard curve (colored)

1 bottle (25 mL) Wash Buffer Concentrate 20x (phosphate-buffered saline with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (12 mL) Sample Diluent (use when an external predilution of the samples is needed)

1 vial (15 mL) Stop Solution (1M phosphoric acid)

2 adhesive Plate Covers

Storage instructions

Store ELISA plate and Standard curves or whole kit at –20°C. The plate and the standard curves can also be removed, stored at –20°C, remaining kit reagents can be stored between 2°C and 8°C. Expiry of the kit and reagents is stated on labels.

The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Sample collection

Cell culture supernatants, serum, and plasma (EDTA, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove the serum or plasma from the clot or red cells as soon as possible after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples must be stored frozen at –20°C to avoid loss of bioactive human MIP-1α. If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (for sample stability refer to "Performance characteristics" on page 4). Avoid repeated freeze-thaw cycles. Prior to assay, frozen serum or plasma should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents and samples

1. Buffer concentrate should be brought to room temperature and diluted before starting the test procedure.
2. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (25 mL) of the Wash Buffer Concentrate (20x) into a clean 500 mL graduated cylinder. Bring to final volume to 500 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2°C to 25°C. The Wash Buffer (1x) is stable for 30 days.

Test protocol

Note:

- Use plate immediately after removal from –20°C!
- Do not wait until pellets have completely dissolved before applying samples; the binding reaction in the standard strips starts immediately after addition of water!
- Do not try to dissolve pellets by pipetting up and down in the wells; some parts of the pellet could stick to the tip creating high variation of results.
- Perform the washing step with at least 400 µL of washing buffer as stated in the manual or fill the wells completely; otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.
- Allow the washing buffer to sit in the wells for a few seconds before aspiration.
- Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

1. Determine the number of microwell strips required to test the desired number of samples plus microwell strips for blanks and standards (colored). Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1).
2. Add distilled water to all standard and blank wells as indicated on the label of the standard strips (A1/A2 to H1/H2).
3. Add 100 µL of distilled water to the sample wells.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 1000.0 pg/mL	Standard 1 1000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 500.0 pg/mL	Standard 2 500.0 pg/mL	Sample 2	Sample 2
C	Standard 3 250.0 pg/mL	Standard 3 250.0 pg/mL	Sample 3	Sample 3
D	Standard 4 125.0 pg/mL	Standard 4 125.0 pg/mL	Sample 4	Sample 4
E	Standard 5 62.5 pg/mL	Standard 5 62.5 pg/mL	Sample 5	Sample 5
F	Standard 6 31.3 pg/mL	Standard 6 31.3 pg/mL	Sample 6	Sample 6
G	Standard 7 15.6 pg/mL	Standard 7 15.6 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 50 µL of each Sample, in duplicate, to the designated wells and mix the contents.
5. Cover with a Plate Cover and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
6. Remove Plate Cover and empty wells. Wash the microwell strips 6 times with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
7. After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.
8. Pipet 100 µL of TMB Substrate Solution to all wells, including the blank wells.
9. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

   The color development on the plate should be monitored and the substrate reaction stopped (see step 10) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

   It is recommended to add the stop solution when the highest standard has developed a dark blue color. The color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
10. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human MIP-1α standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human MIP-1α concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating human MIP-1α for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human MIP-1α concentration.
- Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

  Note: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µL to the final volume per well. These 100 µL are composed of 50 µL of sample diluent plus 50 µL of the sample. This is a 1:2 dilution.

  The remaining 50 µL to give 150 µL are due to the addition of 50 µL conjugate to all wells.

  50 µL sample diluent and 50 µL conjugate results in 50 µL reconstitution volume, addition of 50 µL sample (50 µL + 50 µL = 1:2 dilution).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human MIP-1α levels. Such samples require further external predilution according to expected human MIP-1α values with Sample Diluent in order to precisely quantitate the actual human MIP-1α level.
- It is suggested that each testing facility establishes a control sample of known human MIP-1α concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 4.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

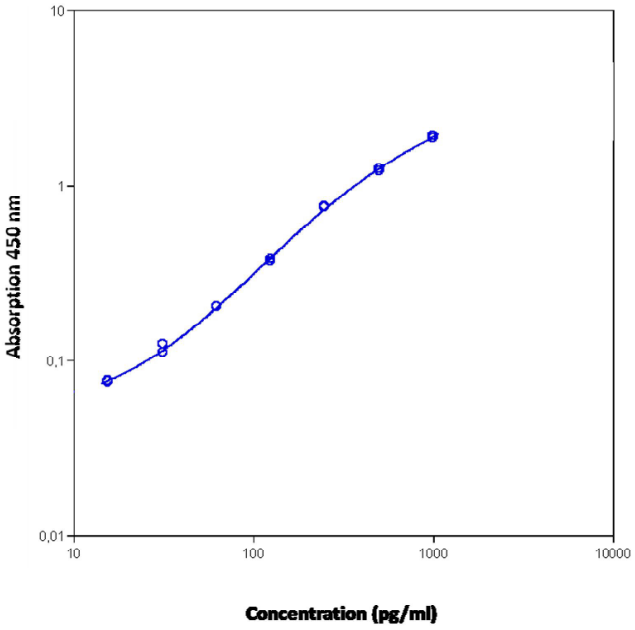

Fig. 4 Representative standard curve for Human MIP-1α Instant ELISA Kit. Human MIP-1α was diluted in serial 2-fold steps in sample diluent, each symbol represents the mean of 3 parallel titrations.

Table 2 Typical data using the Human MIP-1α Instant ELISA Kit.
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human MIP-1α concentration (pg/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	1000.0	1.847 1.925	1.886	2.9
2	500.0	1.253 1.212	1.233	4.6
3	250.0	0.761 0.744	0.753	1.6
4	125.0	0.366 0.379	0.373	2.5
5	62.5	0.204 0.202	0.203	0.7
6	31.3	0.124 0.111	0.118	7.8
7	15.6	0.077 0.075	0.076	1.9
Blank	0.0	0.051 0.050	0.051	1.4

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Because exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks, or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle, and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity

The limit of detection of human MIP-1α defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be <6.0 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human MIP-1α. Two standard curves were run on each plate. Data below show the mean human MIP-1α concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 7.3%.

Table 3 The mean human MIP-1α concentration and the coefficient of variation for each sample.

Positive sample	Experiment	Mean human MIP-1α concentration (pg/mL)	Coefficient of variation (%)
1	1 2 3	463.2 467.6 574.7	7.0 4.0 5.0
2	1 2 3	430.5 420.5 472.0	8.0 5.0 9.0
3	1 2 3	318.4 330.2 319.2	8.0 5.0 8.0
4	1 2 3	198.9 208.1 187.8	7.0 3.0 11.0
5	1 2 3	380.5 381.5 380.6	3.0 4.0 6.0
6	1 2 3	221.9 223.3 214.9	6.0 10.0 9.0
7	1 2 3	99.0 107.5 101.9	7.0 11.0 11.0
8	1 2 3	80.4 88.6 71.4	8.0 10.0 9.0

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human MIP-1α. Two standard curves were run on each plate. Data below show the mean human MIP-1α concentration

and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 5.4%.

Table 4 The mean human MIP-1α concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	Mean human MIP-1α concentration (pg/mL)	Coefficient of variation (%)
1	501.8	12.6
2	441.0	6.2
3	322.6	2.0
4	198.3	5.1
5	380.8	0.1
6	219.8	2.3
7	102.8	4.2
8	80.2	10.7

Spike recovery

The spike recovery was evaluated by spiking 4 levels of recombinant human MIP-1α into human serum. Recoveries were determined in 3 independent experiments with 2 replicates each. The unspiked serum was used as blank in these experiments. The average overall mean recovery was 92.3%.

Dilution parallelism

Four serum samples with different levels of human MIP-1α were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged between 94% and 117% with an overall recovery of 104.4%.

Sample	Dilution	Expected concentration (pg/mL)	Observed concentratio n (pg/mL)	Recovery of exp. val. (%)
1	1:2	–	798.0	–
	1:4	399.0	375.6	94.1
	1:8	187.8	209.8	111.7
	1:16	104.9	105.3	100.3
2	1:2	–	605.9	–
	1:4	303.0	290.8	96.0
	1:8	145.4	149.5	102.8
	1:16	74.7	77.4	103.6
3	1:2	–	434.2	–
	1:4	217.1	234.0	107.8
	1:8	117.0	132.9	113.6
	1:16	66.4	66.6	100.3
4	1:2	–	196.6	–
	1:4	98.3	114.5	116.5
	1:8	57.3	57.9	101.2
	1:16	29.0	30.4	105.0

Sample stability

Freeze-thaw stability

Aliquots of serum samples (unspiked or spiked) were stored at –20°C and thawed 5 times, and the human MIP-1α levels determined. There was no significant loss of human MIP-1α immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human MIP-1α level determined after 72 hours.

There was about 20% loss of MIP-1α immunoreactivity during storage at 4°C, 75% loss at room temperature, and 90% loss at 37°C. Store samples at –20°C!

Specificity

To define the specificity of this ELISA several proteins were tested for cross-reactivity. No cross-reactivity was observed.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20x (25 mL) to 475 mL distilled water.

Test protocol summary

Note: Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

1. Place standard strips in position A1/A2 to H1/H2.
2. Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
3. Add 100 µL distilled water to sample wells.
4. Add 50 µL Sample to designated wells.
5. Cover microwell strips and incubate 3 hours at room temperature (18°C to 25°C) on a microplate shaker.
6. Empty and wash microwell strips 6 times with 400 µL Wash Buffer.
7. Add 100 µL of TMB Substrate Solution to all wells including blank wells.
8. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
9. Add 100 µL Stop Solution to all wells including blank wells.
10. Blank microwell reader and measure color intensity at 450 nm.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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