DRG® MIP-1 beta human (EIA-3788)

REVISED 11 JUNE 2007 **RUO**

INTENDED USE

The DRG Human Macrophage Inflammatory Protein 1 beta (hMIP-1β) ELISA is to be used for the in vitro quantitative determination of hMIP-1β in human serum, plasma (EDTA, citrate, heparin), buffered solution, or cell culture medium. The assay recognizes both natural and recombinant forms of hMIP-1β.

INTRODUCTION

The two variants of the Macrophage Inflammatory Protein 1, designated MIP-1α and MIP-1β, belong to the β or CC chemokine subfamily. The two chemokines were originally purified from lipopolysaccharide-treated murine monocytic cell lines, hence their designation as "Macrophage Inflammatory Protein". MIP-1α and MIP-1β are produced by stimulated leukocytes as well as other tissue cells and tumor cells. At least seven variants of human MIP-1β, corresponding to the products of independently cloned cDNA, including Act-2, G-26, HC-21, HIMAP, H400, pAT 744 and MAD-5, have been described.

At the protein levels, mature human MIP-1β has a length of 69 amino acids and shows a homology of 70% with MIP-1α. MIP-1β is a natural ligand for the chemokine receptors CCR3, CCR5 and CCR8. The effect of MIP-1α and MIP-1β on monocytes and T cells include chemotaxis, a rise in intracellular [Ca^{2+}], expression of integrins and increased adhesion to endothelial cells. The two chemokines provide an important signal for T cell activation resulting in enhanced proliferation, IL-2 secretion and cell surface IL-2 receptor expression. MIP-1α and MIP-1β chemoattract and degranulate NK cells and enhance NK cells as well as CTL mediated cytolysis. Both chemokines induce chemotaxis and [Ca^{2+}] increase in dendritic cells. The two chemokines are reported to be involved in a variety of inflammatory diseases and have been reported as playing a role in CD8+ T cell-mediated HIV suppression.

PRINCIPLE OF THE TEST

The DRG hMIP-1β kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for hMIP-1β has been coated onto the wells of the microtiter strips provided. Samples, including standards of known hMIP-1β content, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the hMIP-1β antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for hMIP-1β is added. During the second incubation, this antibody binds to the immobilized hMIP-1β captured during the first incubation. After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of hMIP-1β present in the original specimen.

WARNINGS AND PRECAUTIONS FOR USERS

General Remarks

- This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Proclin 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.
- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing infectious agents.

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REMARKS FOR THE TEST

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes. The hMIP-1β ELISA kit may be used to measure hMIP-1β in serum, EDTA plasma, citrate plasma, heparinized plasma, buffered solution, or cell culture samples.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls, and samples be run in duplicate.
7. Samples that are >1000 pg/mL should be diluted with Standard Diluent Buffer.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. Do not mix or interchange different reagent lots from various kit lots.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
15. Because Stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between Stabilized Chromogen and metal, or color may develop.

KIT COMPONENTS

Reagents	Quantity 96 Test kit	Quantity 192 Test kit	Preparation and Storage
hMIP-1β Standard, recombinant hMIP-1β. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	Lyophilized 2 – 8°C
Standard Diluent Buffer; Contains 8 mM sodium azide.	1 x 25 mL	2 x 25 mL	2 – 8°C
hMIP-1β Antibody -Coated Wells, 96 wells/plate.	1 plate	2 plate	2 – 8°C
hMIP-1β Biotin Conjugate (Biotin-labeled anti- hMIP-1β), Contains 8 mM sodium azide.	1 x 11 mL	2 x 11 mL	2 – 8°C
Streptavidin-Peroxidase (HRP), (100x), Contains 1.7 mM thymol.	1 x 0.125 mL	2 x 0.125 mL	Concentrate 2 – 8°C
Incubation Buffer, Contains 8 mM sodium azide.	1 x 11 mL	1 x 11 mL	2 – 8°C
Streptavidin-Peroxidase (HRP) Diluent, Contains 1.7 mM thymol and 0.05% Proclin 300.	1 x 25 mL	1 x 25 mL	2 – 8°C
Wash Buffer (25x)	1 x 100 ml	1 x 100 ml	Concentrate 2 – 8°C

Stabilized Chromogen, Tetramethylbenzidine (TMB);	1 x 25 mL	1 x 25 mL	2 – 8°C
Stop Solution	1 x 25 mL	1 x 25 mL	2 – 8°C
Plate Covers, adhesive strips	4	6	2 – 8°C

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtiter plate reader capable of measurement at or near 450 nm.
- Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
- Deionized or distilled H_2O.
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
- Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
- Glass or plastic tubes for diluting and aliquoting standard.
- Absorbent paper towels.
- Calibrated beakers and graduated cylinders in various sizes.

ASSAY PROCEDURE

Directions for Washing

Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Buffer provided. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue. If using an automated washer, the operating instructions for washing equipment should be carefully followed.

PREPARATION AND STORAGE OF REAGENTS

Reconstitution and Dilution of hMIP-1β Standard

The hMIP-1β standard was calibrated against a highly purified E. coli expressed recombinant hMIP-1β.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 5,000 pg/mL with Standard Diluent Buffer. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.100 mL of the reconstituted standard to a tube containing 0.400 mL Standard Diluent Buffer. Label as 1000 pg/mL hMIP-1β. Mix.
3. Add 0.250 mL of Standard Diluent Buffer to each of 6 tubes labeled 500, 250, 125, 62.5, 31.2 and 15.6 pg/mL hMIP-1β.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

Dilution of hMIP-1β Standard

Standard:	Add:	Into:
1000 pg/mL	Prepare as described in Step 2.	
500 pg/mL	0.250 mL of the 1000 pg/mL std.	0.250 mL of the Diluent Buffer
250 pg/mL	0.250 mL of the 500 pg/mL std.	0.250 mL of the Diluent Buffer
125 pg/mL	0.250 mL of the 250 pg/mL std.	0.250 mL of the Diluent Buffer
62.5 pg/mL	0.250 mL of the 125 pg/mL std.	0.250 mL of the Diluent Buffer
31.2 pg/mL	0.250 mL of the 62.5 pg/mL std.	0.250 mL of the Diluent Buffer
15.6 pg/mL	0.250 mL of the 31.2 pg/mL std.	0.250 mL of the Diluent Buffer
0 pg/mL	0.250 mL of the Diluent Buffer.	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.

Storage and Final Dilution of Streptavidin-HRP
Please Note: The Streptavidin-HRP 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow Streptavidin-HRP concentrate to reach room temperature. Gently mix. Pipette Streptavidin-HRP concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µl of this 100x concentrated solution with 1 mL of Streptavidin-HRP Diluent for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused Streptavidin-HRP concentrate to the refrigerator.

Dilution of Wash Buffer
Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY PROCEDURE
Be sure to read the Warnings and Precautions for Users section before carrying out the assay. Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use. **Note:** A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)

2. Add 50 µL of the Standard Diluent Buffer to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of standards, samples or controls to the appropriate microtiter wells. (See PREPARATION AND STORAGE OF REAGENTS, Section 7.2.2.)
4. Add 50 µL of Incubation Buffer to the wells containing standards, serum and plasma samples, or 50 µL of Standard Diluent Buffer to the wells containing cell culture samples.
5. Cover plate with plate cover and incubate for 2 hours at room temperature.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
7. Pipette 100 µL of biotinylated anti-hMIP-1ß (Biotin Conjugate) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
8. Cover plate with plate cover and incubate for 1 hour at room temperature.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
10. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in PREPARATION AND STORAGE OF REAGENTS, Section 7.2.3.)
11. Cover plate with the plate cover and incubate for 30 minutes at room temperature.
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See DIRECTIONS FOR WASHING.
13. Add 100 µL of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.
14. Incubate for 30 minutes at room temperature and in the dark. Please Note: Do not cover the plate with aluminum foil or metalized mylar. The microtiter plate reader used often determines the incubation time for chromogen substrate. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
15. Add 100 µL of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.

CALCULATION OF RESULTS

1. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve-fitting software, the four-parameter algorithm provides the best curve fit.
2. Read the hMIP-1β concentrations for unknown samples and controls from the standard curve plotted in step 1. (Samples producing signals greater than that of the highest standard (1000 pg/mL) should be diluted in Standard Diluent Buffer for serum and plasma samples or corresponding medium for cell culture samples and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0-to 1000 pg/mL hMIP-1β.

hMIP-1β (pg/mL)Standard	Optical Density (450 nm)
0	0.022
	0.026
15.6	0.080
	0.078
31.2	0.142
	0.137
62.5	0.261
	0.253
125	0.473
	0.509
250	0.928
	0.973
500	1.824
	1.778
1000	3.024
	2.972

EXPECTED VALUES

Each laboratory must establish its own normal values. For guidance, the mean of 12 normal sera was 99.7 pg/mL (SD=48.9), (range 24 to 211 pg/mL).
The mean of 12 normal EDTA plasma was 74.6 pg/mL (SD=34.5), (range 26 to 152 pg/mL).
The mean of 12 normal heparinized plasma was 83 pg/mL (SD=50.6), (range 19 to 204 pg/mL).

STIMULATION PROTOCOL

Cell culture supernatants were evaluated in this assay. Human Whole Blood (WB) cells were cultured in RPMI supplemented with 5% FCS for 4, 24, 48 or 72 hours either without stimulation or with a blend of LPS (25 µg/mL) and PHA (5 µg/mL), or with a blend of ionomycin (100 ng/mL) and PMA (100 ng/mL). Results are shown below. Levels of hMIP-1β released from cultured human cells

		hMIP-1β (ng/mL)			
Stimulus	**Cell type**	**4 hrs.**	**24 hrs**	**48 hrs.**	**72 hrs.**
None	WB cells	0.019	0.014	0.015	0.009
LPS + PHA	WB cells	6.4	48.7	50.7	56.5
PMA + ionomycin	WB cells	14.3	27.0	40.1	34.9

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PERFORMANCE CHARACTERISTICS

PRECISION

Intra-Assay Precision

Samples of known hMIP-1ß concentration were assayed in replicates of 20 to determine precision within an assay.

Sample	Sample 1	Sample 2	Sample 3
n	20	20	20
Mean [pg/ml]	72	244.3	574.7
S.D.	1.8	5.1	13.1
%CV	2.5	2.1	2.3

SD = Standard Deviation
CV = Coefficient of Variation

Inter-Assay Precision

Samples were assayed 20 times in multiple assays to determine precision between assays.

Sample	Sample 1	Sample 2	Sample 3
n	20	20	20
Mean [pg/ml]	75.5	240.5	556.7
S.D.	3.8	7.3	18
%CV	5.0	3.0	3.2

SENSITIVITY

The minimum detectable dose of hMIP-1ß is 2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times.

SPECIFICITY

Buffered solutions of a panel of substances at 150 ng/mL were assayed with the DRG hMIP-1ß kit. The following substances were tested and found to have no cross-reactivity: HumanIL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-15, IL-16, IFN-γ, TNF-α, OSM, MIP-1α, MIP-3•, MIP-5, MCP-1, MCP-3, BCA, LIF, M-CSF, GM-CSF, G-CSF, SCF, IP-10, EGF, GRO-α, NAP-2, Eotaxin, Eotaxin-2, RANTES; mouse MIP-1β, Eotaxin; rat IL-1β, IL-2, IL-4, IL-6, IL-10, MIP-2, CINC-2β, TNF-α.

RECOVERY

The recovery of hMIP-1β added to human serum averaged 100%. In plasma, hMIP-1β recoveries averaged 99% (EDTA), 93% (citrate) and 88% (heparin). The recovery of hMIP-1β added to tissue culture medium containing 10% fetal calf serum averaged 93%.

LINEARITY OF DILUTION

Human serum containing 904 pg/mL of measured hMIP-1ß was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

	Serum		
Dilution	**Measured (pg/mL)**	**Expected (pg/mL)**	**% Expected**
Neat	904	--	--
1/2	431	452	95
1/4	216	226	96
1/8	116	113	102
1/16	58	56.5	102
1/32	29	28.3	102

PARALLELISM

Natural hMIP-1β was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism between the natural hMIP-1β and the standard protein was demonstrated and indicated that the standard accurately reflects natural hMIP-1β content in samples.

LIMITATIONS OF PROCEDURE

Do not extrapolate the standard curve beyond the 1000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >1000 pg/mL with Standard Diluent Buffer, reanalyze these and multiply results by the appropriate dilution factor. The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native hMIP-1ß in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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