



ELISA Kit

Catalog # **KAC2201 (96 tests)**

*Human*  
**MIP-1 $\alpha$**

[www.invitrogen.com](http://www.invitrogen.com)

Invitrogen Corporation

542 Flynn Road, Camarillo, CA 93012

Tel: 800-955-6288

E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)



# Table of Contents

Table of Contents.....	3
Contents and Storage.....	4
<b>Introduction.....</b>	<b>5</b>
Purpose.....	5
Principle of the Method.....	5
<b>Methods.....</b>	<b>6</b>
Materials Needed But Not Provided.....	6
Procedural Notes.....	6
Preparation of Reagents.....	7
Assay Procedure.....	8
Typical Data.....	9
<b>Performance Characteristics.....</b>	<b>10</b>
Sensitivity.....	10
Precision.....	10
Linearity of Dilution.....	10
Recovery.....	11
Specificity.....	11
Limitations of the Procedure.....	11
<b>Appendix.....</b>	<b>12</b>
Troubleshooting Guide.....	12
Technical Support.....	13
References.....	14
Citations.....	14

## Contents and Storage

---

**Storage**      Store at 2 to 8°C.

---

### Contents

Reagents Provided	96 Test Kit
<i>Hu MIP-1α Standard</i> , lyophilized, recombinant <i>Hu MIP-1α</i> . Refer to vial label for quantity and reconstitution volume.	2 vials
<i>Standard Diluent Buffer</i> . Contains 7.7 mM sodium azide; 25 ml per bottle.	1 bottle
<i>Hu MIP-1α Controls</i> (two levels), recombinant <i>Hu MIP-1α</i> in plasma matrix, lyophilized. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.	2 vials
<i>Hu MIP-1α Antibody-Coated Wells</i> , 96 wells per plate.	1 plate
<i>Hu MIP-1α Biotin Conjugate</i> (Biotin-labeled anti- <i>Hu MIP-1α</i> ). Contains 7.7 mM sodium azide; 7 ml per bottle.	1 bottle
<i>Streptavidin-Peroxidase (HRP)</i> , (100x concentrate). Contains 0.04% Proclin® 300 and 3.3 mM thymol; 0.125 ml per vial.	1 vial
<i>Streptavidin-Peroxidase (HRP) Diluent</i> . Contains 0.04% Proclin® 300 and 3.3 mM thymol; 25 ml per bottle.	1 bottle
<i>Wash Buffer Concentrate (25X)</i> . 100 mL per bottle.	1 bottle
<i>Stabilized Chromogen, Tetramethylbenzidine (TMB)</i> . 25 mL per bottle.	1 bottle
<i>Stop Solution</i> . 25 mL per bottle.	1 bottle
<i>Plate Covers</i> , adhesive strips.	3

---

**Disposal Note**      This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Proclin® 300 is toxic. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. 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.

---

**Safety**      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 of infectious agents.

---

# Introduction

---

## Purpose

The Invitrogen Human Macrophage Inflammatory Protein-1 alpha (Hu MIP-1 $\alpha$ ) ELISA is to be used for the quantitative determination of Hu MIP-1 $\alpha$  in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Hu MIP-1 $\alpha$ .

**For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.**

---

## Principle of the Method

The Invitrogen Hu MIP-1 $\alpha$  kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). An antibody specific for Hu MIP-1 $\alpha$  has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu MIP-1 $\alpha$  content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated secondary antibody.

During the first incubation, the Hu MIP-1 $\alpha$  antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

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 second 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 Hu MIP-1 $\alpha$  present in the original specimen.

---

## Background Information

Macrophage Inflammatory Protein-1 alpha (MIP-1 $\alpha$ ) is an endogenous fever-inducing substance that binds heparin and is resistant to cyclooxygenase inhibition. Macrophages stimulated by endotoxin may secrete this protein, termed MIP-1 $\alpha$ , which differs from tumor necrosis factor (TNF) and IL-1, as well as other endogenous pyrogens because its action is not associated with prostaglandin synthesis. It appears indistinguishable from hematopoietic stem cell inhibitor and may function in growth regulation of hematopoietic cells.

MIP-1 $\alpha$  is chemoattractive to monocytes, neutrophils, eosinophils, dendritic cells, NK and T cells. Interestingly, MIP-1 $\alpha$  exerts a potent anti-tumor effect because of its ability to recruit immune cells to the tumor site. Indeed, tumor cells engineered to express MIP-1 $\alpha$  possess a reduced tumorigenicity *in vivo*, an effect attributed to the recruitment of macrophages and neutrophils at the tumor site. MIP-1 $\alpha$  also elicited a long-term immune response which resulted in the protection of the animals against challenge with the parental tumor cells (1).

The growth of pulmonary metastases is inhibited by transfection of MIP-1 $\alpha$  into B16F10 melanoma cells. MIP-1 $\alpha$  is effective in preventing the initiation of metastasis but not at sustaining an effective anti-tumor response (2).

Langerhans cells are the major source of mRNA for IL-1 $\beta$  and MIP-1 $\alpha$  among unstimulated mouse epidermal cells (3).

Polymerization of murine MIP-1 $\alpha$  inactivates its myelosuppressive effects *in vitro*: the active form is a monomer (4).

The chemokine MIP-1 $\alpha$  and its receptor CCR1 control pulmonary inflammation and antiviral host defense in paramyxovirus infection (5).

# Methods

---

## Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
  - Calibrated adjustable precision pipettes
  - Distilled or deionized water
  - Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
  - Glass or plastic tubes for diluting solutions
  - Absorbent paper towels
  - Calibrated beakers and graduated cylinders
- 

## Procedural Notes

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 to 8°C to maintain plate integrity.
  3. Samples should be collected in pyrogen/endotoxin-free tubes.
  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. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
  8. **Do not mix or interchange different reagent lots from various kit lots.**
  9. Do not use reagents after the kit expiration date.
  10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
  11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
  12. 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.
  13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.
- 

## Directions for Washing

- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.
  - Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip 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 Buffer*. 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 diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *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, follow the washing instructions carefully.
-

# Preparation of Reagents

## Dilution of Standard

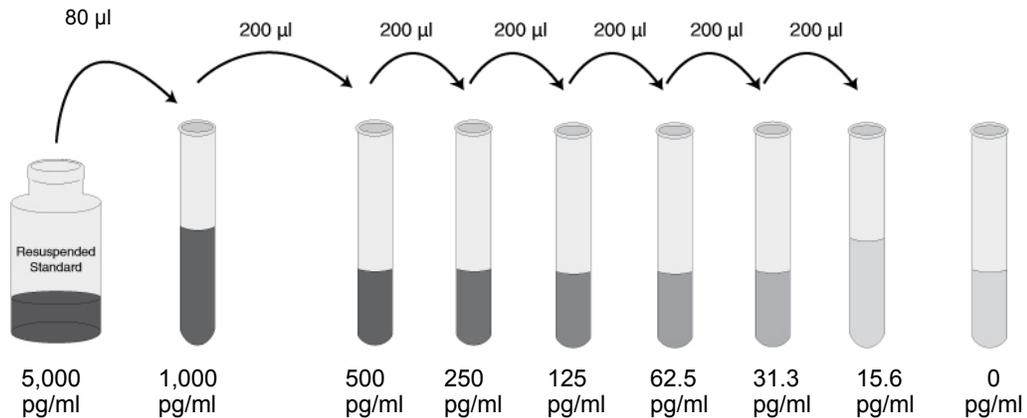
**Note:** Either glass or plastic tubes may be used for standard dilutions.

1 pg of standard preparation is equivalent to 0.25 mU WHO reference preparation 92/518 (NIBSC, Hertfordshire, UK, EN6 3QG).

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.
2. Add 0.080 ml of the reconstituted standard to a tube containing 0.320 ml *Standard Diluent Buffer*. Label as 1,000 pg/ml Hu MIP-1 $\alpha$ . Mix.
3. Add 0.200 ml of *Standard Diluent Buffer* to each of 6 tubes labeled 500, 250, 125, 62.5, 31.3 and 15.6 pg/ml Hu MIP-1 $\alpha$ .
4. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

## Note

Remaining reconstituted standard should be discarded. Return the *Standard Diluent Buffer* to the refrigerator.



## Preparing SAV-HRP

**Note: Prepare within 15 minutes of usage.** The *Streptavidin-HRP* (100x concentrate) is in 50% glycerol, which 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.
2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

# 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

---

## Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* 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.
  2. 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 *Procedural Notes* 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  $\mu$ l of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50  $\mu$ l of standards, samples or controls to the appropriate microtiter wells. See **Preparations of Reagents**.
4. Pipette 50  $\mu$ l of biotinylated anti-Hu MIP-1 $\alpha$  (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
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. Add 100  $\mu$ l Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Direction for Washing**.
10. Add 100  $\mu$ l of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**. **Note: Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. 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 exceeds 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.
12. Add 100  $\mu$ l of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100  $\mu$ l each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.

14. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
  15. Read the concentrations for unknown samples and controls from the standard curve. (Samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)
- 

**Typical  
Data  
(Example)**

The following data were obtained for the various standards over the range of 0 to 1,000 pg/ml Hu MIP-1 $\alpha$ .

Standard Hu MIP-1 $\alpha$ (pg/ml)	Optical Density (450 nm)
1,000	3.33
500	1.60
250	0.79
125	0.43
62.5	0.23
31.3	0.13
15.6	0.08
0	0.03

---

# Performance Characteristics

---

**Sensitivity** The minimum detectable dose of Hu MIP-1 $\alpha$  is 2 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

---

**Precision** 1. Intra-Assay Precision  
Samples of known Hu MIP-1 $\alpha$  concentration were assayed in replicates of 32 to determine precision within an assay.

	Sample 1	Sample 2
Mean (pg/ml)	105	290
SD	8.0	16.0
%CV	7.6	5.5
SD = Standard Deviation CV = Coefficient of Variation		

2. Inter-Assay Precision  
Samples were assayed 40 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2
Mean (pg/ml)	259	822
SD	19.0	56.0
%CV	7.3	6.8
SD = Standard Deviation CV = Coefficient of Variation		

**Linearity of Dilution** A human serum pool containing 15 ng/ml of measured Hu MIP-1 $\alpha$  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.

---

---

<b>Recovery</b>	The recovery of Hu MIP-1 $\alpha$ added to pooled normal human serum averaged 90% (range: 81% to 99%). The recovery of Hu MIP-1 $\alpha$ added to tissue culture medium containing 10% fetal bovine serum averaged 110% (range: 100% to 113%).
<b>Specificity</b>	Buffered solutions of a panel of substances at 150 ng/ml were assayed with the Invitrogen Hu MIP-1 $\alpha$ kit. The following substances were tested and found to have no cross-reactivity: human GRO, IP-10, MCP-1, RANTES, MIP-1 $\alpha$ , MCP-3, MCP-4, MIP-5, IL-8 and NAP-2.
<b>Limitations of the Procedure</b>	<p>Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with <i>Standard Diluent Buffer</i>; reanalyze these and multiply results by the appropriate dilution factor.</p> <p>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 Hu MIP-1<math>\alpha</math> 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.</p>

---

# Appendix

---

## Troubleshooting Guide

---

Elevated background

*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing either biotin or SAV-HRP can elevate the background if residual is left in the well.

*Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.

*Cause:* Contamination of substrate solution with metal ions or oxidizing reagents.

*Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

*Cause:* Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate.

*Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

*Cause:* Incubation time is too long or incubation temperature is too high.

*Solution:* Reduce incubation time and/or temperature.

---

Elevated sample/standard ODs

*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.

*Solution:* Follow the protocol instructions regarding the dilution of the standard.

*Cause:* Incorrect dilution of the SAV-HRP conjugate.

*Solution:* Warm solution of SAV-HRP concentrate to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in SAV diluent provided.

*Cause:* Incubation times extended.

*Solution:* Follow incubation times outlined in protocol.

*Cause:* Incubations carried out at 37°C when RT is dictated.

*Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

---

Poor standard curve

*Cause:* Improper preparation of standard stock solution.

*Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample.

*Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted.

*Solution:* NEVER substitute any components from another kit.

*Cause:* Errors in pipetting the standard or subsequent steps.

*Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

---

Weak/no  
color  
develops

*Cause:* Reagents not at RT ( $25 \pm 2^\circ\text{C}$ ) at start of assay.

*Solution:* Allow ALL reagents to warm to RT prior to commencing assay.

*Cause:* Incorrect storage of components, e.g., not stored at 2 to  $8^\circ\text{C}$ .

*Solution:* Store all components exactly as directed in protocol and on labels.

*Cause:* Working SAV-HRP solution made up longer than 15 minutes before use in assay.

*Solution:* Use the diluted SAV-HRP within 15 minutes of dilution.

*Cause:* TMB solution lost activity.

*Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded.

*Solution 2:* Avoid contact of the TMB solution with items containing metal ions.

*Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.

*Solution:* Please contact Technical Support for advice when using nonvalidated sample types.

*Cause:* Wells have been scratched with pipette tip or washing tips.

*Solution:* Use caution when dispensing and aspirating into and out of microwells.

Poor  
Precision

*Cause:* Errors in pipetting the standards, samples or subsequent steps.

*Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.

*Cause:* Repetitive use of tips for several samples or different reagents.

*Solution:* Use fresh tips for each sample or reagent transfer.

*Cause:* Wells have been scratched with pipette tip or washing tips.

*Solution:* Use caution when dispensing and aspirating into and out of microwells.

---

## Technical Support

---

### Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to [www.invitrogen.com/ELISA](http://www.invitrogen.com/ELISA).



#### USA:

Invitrogen Corporation  
542 Flynn Road  
Camarillo, CA 93012

Tel: 800-955-6288

E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

#### Europe:

Invitrogen Ltd  
Inchinnan Business Park  
3 Fountain Drive  
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100

Fax: +44 (0) 141 814 6117

E-mail: [eurotech@invitrogen.com](mailto:eurotech@invitrogen.com)

---

---

## References

1. Nakashima, E., et al. (1996) A candidate for cancer gene therapy: MIP-1 alpha gene transfer to an adenocarcinoma cell line reduced tumorigenicity and induced protective immunity in immunocompetent mice. *Pharm. Res.* (12):1896-1901.
2. van Deventer, H.W., et al. (2002) Transfection of macrophage inflammatory protein 1 alpha into B16 F10 melanoma cells inhibits growth of pulmonary metastases but not subcutaneous tumors. *J. Immunol.* 169(3):1634-1639.
3. Matsue, H., et al. (1992) Langerhans cells are the major source of mRNA for IL-1 beta and MIP-1 alpha among unstimulated mouse epidermal cells. *J. Invest. Dermatol.* 99(5):537-541.
4. Mantel, C., et al. (1993) Polymerization of murine macrophage inflammatory protein 1 alpha inactivates its myelosuppressive effects in vitro: the active form is a monomer. *Proc. Natl. Acad. Sci. (USA)* 90(6):2232-2236.
5. Domachowske, J.B., et al. (2000) The chemokine macrophage-inflammatory protein-1 alpha and its receptor CCR1 control pulmonary inflammation and antiviral host defense in paramyxovirus infection. *J. Immunol.* 165(5):2677-2682.

---

## Citations

1. Hale-Donze, H., et al. (2002) *J. Immunol.* 169(7):3854-3862.
2. Nardelli, B., et al. (2002) *J. Immunol.* 169(9):4822-4830.
3. Sriwanthana, B., et al. (2001) *AIDS Res. Hum. Retroviruses* 17:719-734.

For an up-to-date and complete list, visit [www.invitrogen.com/ELISA](http://www.invitrogen.com/ELISA) or contact Technical Support.

---

## Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Support Representatives. Invitrogen warrants that all of its products will perform according to the specifications stated on the Certificate of Analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

---

## Licensing Information

These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, [www.invitrogen.com](http://www.invitrogen.com)). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

---

## Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Copyright © Invitrogen Corporation. 09 February 2010

# Human MIP-1 $\alpha$ Assay Summary

Add 50  $\mu$ L of standards, controls & samples

Add 50  $\mu$ L of Biotin Conjugate  
Incubate for 2 hours at RT

aspirate and wash 4x

Incubate 100  $\mu$ L of Streptavidin-HRP  
Working Solution for 30 minutes at RT

aspirate and wash 4x

Incubate 100  $\mu$ L of Stabilized Chromogen  
for 30 minutes at RT

Add 100  $\mu$ L of Stop Solution  
and read at 450 nm

Total time: 3 hours

