Enzyme Immunoassay for
Total Human MIC-1 / NAG–1 / GDF-15

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INTRODUCTION

Macrophage inhibitory cytokine (MIC-1) is a divergent member of the TGF-β superfamily. The cDNA sequence of MIC-1 is identical with several other sequences, including growth differentiation factor-15 (GDF-15), placental bone morphogenetic protein (PLAB), placental transforming growth factor (PTGF -β), prostate-derived factor (PDF), and nonsteroidal anti-inflammatory drug-activated protein-1 (NAG-1). MIC-1 mRNA encodes a secreted protein, resulting from cleavage of a propeptide to give rise to the mature form as a 25-kDa homodimer, which contains seven conserved cysteine residues in the carboxyl terminal. There are at least two known alleles of MIC-1 that are due to a G C point substitution at position 6 of the mature protein which alters a histidine to an aspartic acid (1).

MIC-1 is distributed in various tissues, being highly expressed in macrophages, choroid plexus, prostate, lung, kidney proximal tubules, placenta and intestinal mucosa. It is poorly expressed in the heart although it has been described as a prognostic marker in acute myocardial infarction (2) as well as an independent predictor of chronic heart disease mortality (3).

Initially, MIC-1 was considered to function primarily as a macrophage inhibitor, but recent studies suggest that it is pleiotropic regulating a myriad of cellular processes such as the cell cycle, proliferation, differentiation, and apoptosis. MIC-1 expression can be induced by stress conditions such as tissue injury, malignancy and inflammation. It has recently been implicated as a cachexia mediator inducing weight loss (4).

MIC-1 is overexpressed by a variety of cancers, which may relate to its antitumorigenic and proapoptotic properties, although recent studies describe contradictory mechanisms. For example, it has been reported to exhibit both tumorigenic and antitumorigenic activities. MIC-1 expression is correlated with the tumorigenicity of melanoma cells where it is highly expressed (5). MIC-1 may serve as a biomarker for the prediction of gastric cancer progression. Serum concentrations in cancer patients were 10-fold higher than those of healthy controls (6). Serum MIC-1 has been described as a biomarker capable of predicting prostate cancer prognosis (7).

Prostate-derived factor (PDF/MIC-1) may be related to cellular stress through its interaction with p53. The p53 tumor suppressor modulates cellular responses in various models of cell stress. Furthermore, there appears to be a requirement for functional p53 in PDF induction in these disparate models indicating that PDF may represent a novel target of p53 in response to cell stress (8).

PRINCIPLES OF PROCEDURE

This assay is a quantitative sandwich enzyme immunoassay. Plates are precoated with a polyclonal antibody specific for native human MIC–1 (capture antibody). The antibody-bound MIC-1 in standards and specimens binds to a polyclonal detection antibody. An HRP conjugated anti-detection signal antibody is added followed by substrate. Color development is stopped at the appropriate time and the plates are read. Total assay time is approximately 4 hours.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	96-well microplate coated with anti-MIC-1	1 plate	4°C	GF10a
Assay Buffer	Buffer used to dilute kit reagents and samples	100 mL	4°C	GF10b
10x Wash Buffer	10x solution for washing the plate	30 mL	4°C	GF10c
MIC-1 Standard	Purified native human MIC-1 (2000 pg/mL)	60 µL	-70°C	GF10d
Detection Antibody	Anti-human MIC-1	130 µL	-70°C	GF10e
HRP Conjugate	Anti IgG-HRP conjugate	130 µL	-70°C	GF10f
TMB Substrate	Stabilized TMB substrate	25 mL	4°C	GF10g

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with 450 nm filter
2. Adjustable micropipettes and tips
3. 3 N Sulfuric Acid (H_2SO_4)
4. Deionized Water (dH_2O)
5. Phosphate Buffered Saline (PBS)

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended and they may compromise the performance of the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein, e.g. the use of gloves.

PROCEDURAL NOTES

1. Reagents can be used immediately upon removal from refrigeration.
2. Performance of the entire plate at once is not required. When performing a portion of this kit, return the kit to 4°C. The unused portions of the microplate should be returned to the zip lock pouch with desiccant, and as the antibodies are most stable at the stock concentrations provided, use only the appropriate amount of the stock and store the remainder for subsequent uses.
3. To minimize error due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel.

SAMPLE COLLECTION AND PREPARATION

This kit is designed for the analysis of biological fluids such as serum, plasma, cell culture supernant and urine. Samples should be diluted with assay buffer as part of the reagent prep just prior to running an assay. Recommended starting dilutions are neat or 1:2 with Assay Buffer. Urine may require higher dilutions.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the wash buffer 1:10 by adding 30 mL of 10x Wash Buffer to 270 mL of dH_2O.
2. **MIC-1 Standard:** Immediately prior to use, thaw the MIC-1 Standard (40 ng/mL) and prepare a 1:20 dilution using assay buffer by adding 50 ul of the 40 ng/mL stock to 950 uL of assay buffer. After the dilution the concentration will be 2,000 pg/mL.
3. **Detection Antibody:** Immediately prior to use, dilute 1:100 by adding 120 μL of Detection Antibody to 12 mL of Assay Buffer.
4. **HRP-Conjugate:** Immediately prior to use, dilute 1:100 by adding 120 μL of HRP-Conjugate to 12 mL of Assay Buffer.

STANDARD PREPARATION

Set up for the standard curve by labeling dilution tubes and dispensing the indicated volumes of Assay Buffer according to Table 1 below. Dispense the specified volume of the MIC-1 Standard (2000 pg/mL) Solution.

Table 1: Standard Curve Preparation

Standard	Human MIC-1 (pg/mL)	Assay Buffer (μL)	2000 pg/mL MIC-1 Standard Solution (μL)	Final Volume (μL)
S_7	2000	---	500	500
S_6	1000	250	250	500
S_5	500	375	125	500
S_4	250	437.5	62.5	500
S_3	100	475	25	500
S_2	50	487.5	12.5	500
S_1	20	495	5	500
S_0	0	500	---	500

ASSAY PROCEDURE

1. Add 100 μL of Standards or diluted unknowns to each well in duplicate. See Scheme I for a suggested plate layout. Incubate at room temperature for one hour.
2. Wash wells according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 µL of 1x Wash Buffer.
   d. Let stand for 2 minutes and tap out the contents of the well.
   e. Repeat wash two more times.
3. Add 100 μL of the diluted Detection Antibody per well. Incubate for one hour at room temperature.
4. Wash according to step 2.
5. Add 100 μL of diluted HRP-Conjugate per well. Incubate for one hour at room temperature.
6. Wash according to step 2.
7. Add 150 μL TMB Substrate per well and allow the color to develop for 30 minutes at room temperature.
8. Add 50 µL of 3 M Sulfuric Acid (H_2SO_4) to each well to stop the reaction.
9. Read the plate at 450 nm in a microplate reader.

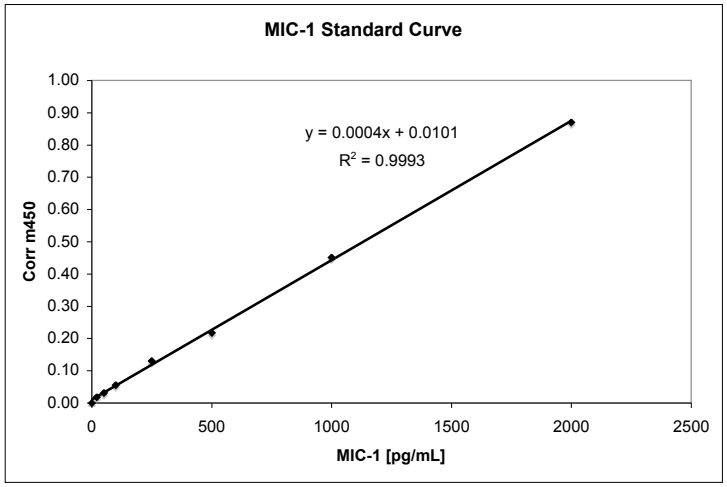
Scheme I: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_1	S_1	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_2	S_2	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_3	S_3	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_5	S_5	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_6	S_6	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	S_7	S_7	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	RB	RB

CALCULATIONS

1. Average all duplicate well absorbance values.
2. Subtract the average absorbance values for the blank wells (S_0) from all other well pairs.
3. Plot a standard curve using the corrected absorbance values of each Standard (y-axis) versus the Standard concentration (x-axis).
4. Determine the concentration of each unknown using the equation of the line.

Figure 1: Typical Standard Curve



EXPECTED VALUES

The following ranges of MIC-1 have been observed with this assay:

Serum and Plasma: 85 – 1350 pg/mL

Urine: 34 – 32,000 pg/mL

REFERENCES

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