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NWLSS™ Macrophage Inhibitory Cytokine MIC-1 ELISA

Product NWK-MIC1H1

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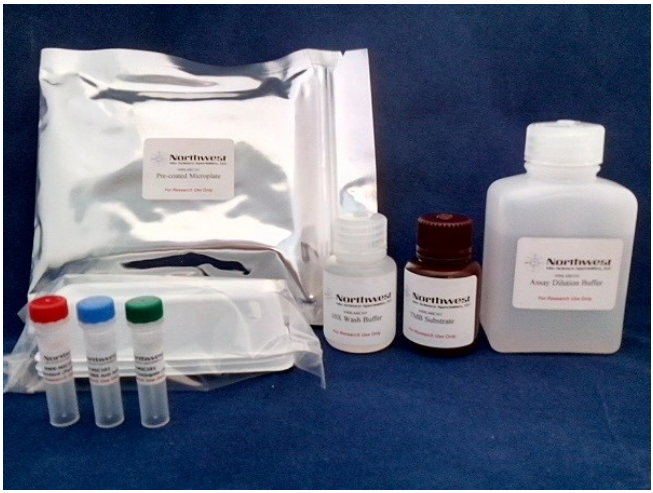

Assay system for measurement of Macrophage Inhibitor Cytokine (MIC-1) in biological fluids and extracts where MIC-1 may be present.

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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).

Intended Use:

This kit is intended for the quantification of macrophage inhibitory cytokine (MIC-1) in serum, plasma, tissue and cell lysates and other biological fluids where MIC-1 may be present.

Test Principle:
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.

General Specifications:

Format:	96 well sandwich ELISA
Number of tests:	Triplicate = 24 Duplicate= 40
Specificity:	Human Macrophage Inhibitory Factor (MIC-1)
Sensitivity:	5 pg/mL
Effective Range:	5 pg/mL - 2000 pg/mL

Kit Contents

Microwells precoated with anti human MIC-1 antibody:	1 X 96 wells
MIC-1 Standard: Purified MIC-1 (40 ng/mL)	1 X 60 µL
Primary Antibody (100X Anti human MIC-1):	1 X 130 µL
10X Wash Buffer:	1 X 30 mL
Assay Dilution Buffer:	1 X 100 mL
TMB Substrate:	1 X 25 mL
Anti IgG-HRP Conjugate (100X):	1 X 130 µL

Required Materials Not Provided:
Adjustable pipettes with a range of 10 µL to 1,000 µL with disposable tips.

Glassware for reagent preparation.

Deionized water.

3M sulfuric acid.

Required Instrumentation:
Microtiter plate reader with 450 nm capability.

Warnings, Limitations, Precautions:
Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage Instructions:
Store the Standard, 100X Primary Antibody and 100X IgG-HRP Enzyme Conjugate at –20°C. All other components should be stored at 4°C. The kit is stable for 9 months from date of manufacture when stored under these conditions.

Assay Preparation
1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate.

2. Create an assay template showing positioning of standards, controls and samples. Include blank wells also.

3. Next remove the required number of strips and place in the frame supplied. Return unused wells to the storage bag, seal and store at 2-8ºC.

Reagent Preparation:
The following instructions are based on the user using the entire kit at one time.

Assay Dilution Buffer is supplied ready to use.

TMB Substrate is supplied ready to use.

10X Wash Buffer
Add the contents of the 10X Wash Buffer to 270 mL deionized H_2O, mix well and label as **Working Wash Buffer.**

100X IgG-HRP Enzyme Conjugate
Briefly centrifuge the vial to remove all liquid from the cap and vial walls. Add 120 µL conjugate to 12 mL Assay Dilution Buffer. Label as **Diluted HRP-Conjugate.**

100X Primary Antibody
Add 120 µL Primary Anti–MIC-1 Antibody to 12 mL Assay Dilution Buffer. Label as **Diluted Primary Antibody**.

Standard Preparation:
1. Label tubes 1-8 tubes as:
2000, 1000, 500, 250, 125, 62.5, 31.25 and zero (0) pg/mL.

2. Add 950 µL ***Assay Dilution Buffer*** to tube 1 and 500 µL **Assay Dilution Buffer** to each of tubes 2-8.

3. Add 50 µL ***of 40 ng/mL MIC-1 Standard*** to tube 1 and mix well.
Note: Unused reconstituted standard can be frozen at -70 °C and thawed one time only without significant loss of immunoreactivity.

4. Make a serial dilution by transferring 500 µL of 2000 pg/mL Standard (tube 1) into tube 2 mixing thoroughly then 500 µL of resulting 1000 pg/mL to tubes 3 and so on to create all Standards down to 31.25 pg/mL.

Sample Handling/Preparation
Samples should be diluted with Assay Dilution Buffer just prior to assay. Recommended starting dilutions are neat and 1:2 with Assay Dilution Buffer. Urine may require additional dilution. 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

Assay Protocol:
1. Add 100 µL of **Standard** or **Sample** to each well. Incubate for 1 hour at room temperature.

2. Wash plate with 300µL **Working Wash Buffer** 3 times allowing plate to stand 2 minutes per wash. Empty plate again by inversion and pat dry upside-down on a lint free towel after final wash.

3. Add 100 µL of **Diluted Primary Antibody** to each well. Incubate for 1 hour at room temperature.

4. Wash according to Step 2.

5. Add 100 µL of **Working HRP-Conjugate** per well. Incubate for 1 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 20-40 minutes at room temperature.

8. Add 50 µL of 3M sulfuric acid to each well to stop the reaction.

9. Record the absorbance at 450 nm using a plate reader.

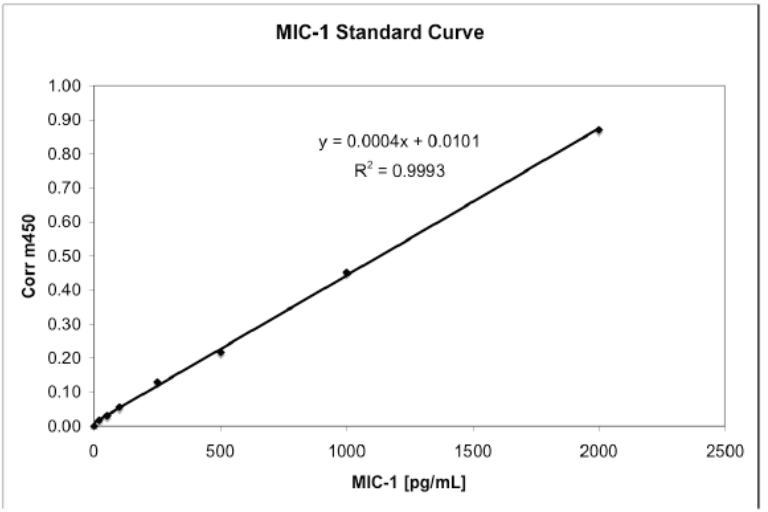
Data Analysis

1. Average all duplicate well absorbance values.

2. Subtract the average absorbance values for the blank wells (S0) 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.



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User Notes:

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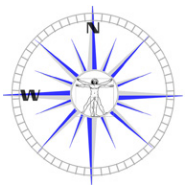

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