

Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Human IL-16 Assay
Ultra-Sensitive Kit

1-Plate Kit	K151JIC-1
5-Plate Kit	K151JIC-2
25-Plate Kit	K151JIC-4

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MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Human IL-16 Assay

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

Ordering information

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Introduction

introduction

Interleukin-16 (IL-16) is produced by epithelial cells,^[1] lymphocytes,^[2] macrophages,^[3] eosinophils,^[4] mast cells,^[4] synovial fibroblasts,^[5] keratinocytes and monocyte-derived dendritic cells (DC).^[6] IL-16 is synthesized as a precursor which is then cleaved by caspase-3 to a 20 kDa mature and biologically active multimer molecule.^[7] The predicted amino acid sequence for precursor or secreted IL-16 does not contain a signal peptide required for the transport of the molecule to the endoplasmic reticulum. The mechanism by which IL-16 is secreted has not yet been elucidated. IL-16 mRNA is constitutively expressed in both CD4⁺ and CD8⁺ cells, and the cytokine is released in response to antigen, mitogen, histamine and serotonin.^[8]

In comparison to other interleukins, IL-16 mRNA expression is almost entirely limited to lymphatic tissues underlining the potential of IL-16 as an immunomodulatory molecule that may play an important role in recruitment and activation of immune cells to the site of inflammation. IL-16 was described and characterized as the first lymphocyte chemotactic factor.^[9] As the soluble ligand for CD4, it was initially depicted as a chemoattractant with specificity for CD4⁺ T cells but later it was determined as a potent chemoattractant for eosinophils, monocytes and monocyte-derived DCs.^[6, 8] Besides its chemotactic properties IL-6 upregulates IL-2R α and activates CD4⁺ T cells synergistically with IL-2 and IL-15.^[10]

Mature IL-16 has many more biological activities such as involvement in cell cycle progression of T cells,^[11] induction of early gene phosphorylation^[12] and pro-B cell differentiation.^[13] In addition, IL-16 is involved in the synthesis of proinflammatory cytokines, including IL-1 β , IL-6 and tumor necrosis factor alpha (TNF- α) in monocytic cells.^[14]

Abnormal levels of IL-16 have been reported in atopic dermatitis,^[15] multiple sclerosis,^[16] asthma,^[1] systemic lupus erythematosus,^[17] rheumatoid arthritis^[18] and other inflammatory diseases such as inflammatory bowel disease,^[19] Crohn's disease and colitis.^[20] IL-16 has been shown to play a role as an inhibitor of immunodeficiency virus replication.^[21,22] As such, IL-16 represents an important cytokine biomarker for the study of human disease and potential therapies.

Principle of the Assay

principle of the assay

MSD[®] assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and multiplex formats. The antibody for a specific protein target is coated on one electrode (or “spot”) per well. The Human IL-16 Assay detects IL-16 in a sandwich immunoassay format (Figure 1). For this assay, MSD provides a Cytokine Panel 14 plate that has been pre-coated with capture antibodies on spatially distinct spots. The position of IL-16 capture antibody is indicated in Figure 1 and on the plate packaging. The user adds the sample and a solution containing the labeled detection antibody— anti-IL-16 labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. IL-16 in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound IL-16 completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR[®] instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of IL-16 present in the sample.

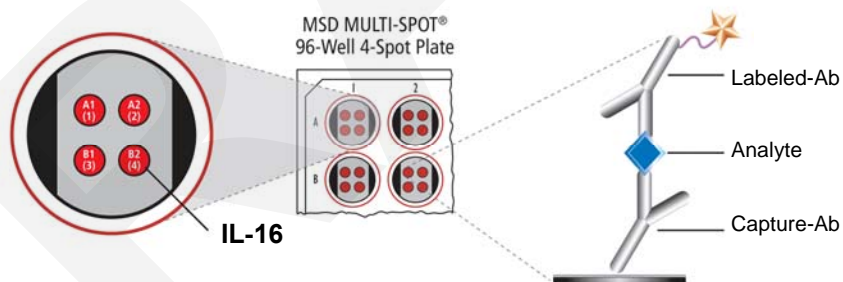


Figure 1. Spot diagram showing placement of analyte capture antibody on Cytokine Panel 14 Plate. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K151JIC-1	K151JIC-2	K151JIC-4
MULTI-SPOT [®] 96-well 4 Spot Cytokine Panel 14 US Plate(s) N45036A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG [™] Anti-hIL-16 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human IL-16 Calibrator (2 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 2 R51BB-4 (8 mL) R51BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 3 R51BA-4 (5 mL) R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (200 mL ea)

IV Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

V Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Important: Upon first thaw, separate Diluent 2 and Diluent 3 into aliquots appropriate to the size of your assay needs. These diluents can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Calibrator and Control Solutions

Calibrator for the Human IL-16 assay is supplied at 400-fold higher concentration than the recommended highest calibrator. Prepare a diluted stock Calibrator by diluting the stock Calibrator 100-fold in Diluent 2. MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 25 μ L of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 2 alone for the 8th point:

Standard	IL-16 (pg/mL)	Dilution Factor
100X Stock	2000000	
STD-01	20000	100
STD-02	5000	4
STD-03	1250	4
STD-04	313	4
STD-05	78	4
STD-06	20	4
STD-07	4.9	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

- 1) Prepare the diluted stock Calibrator by transferring 10 μ L of the Human IL-16 stock Calibrator to 990 μ L Diluent 2.
- 2) Prepare the highest Calibrator point (STD-01) by transferring 50 μ L of the Human IL-16 diluted stock Calibrator to 150 μ L Diluent 2. Repeat 4-fold serial dilutions 6 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 2 (i.e. zero Calibrator).

Notes:

- a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.
- b. The standard curve can be modified as necessary to meet specific assay requirements.

Dilution of Samples

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Serum and plasma samples may be run neat in the MSD Human IL-16 Assay.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Human IL-16 Assay. If using serum-free medium, the presence of carrier protein (e.g., 1% BSA) in the solution is helpful to prevent loss of analyte to the labware. Samples from experimental conditions with extremely high levels of cytokines may require a dilution.

Other Matrices

Information on preparing samples in other matrices, including sputum, CSF, and tissue homogenates can be obtained by contacting MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 50X stock of Anti-hIL-16 Antibody. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 μ L aliquot of the stock Anti-hIL-16 Antibody into 2.94 mL of Diluent 3.

Prepare Read Buffer

The Read Buffer should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of 4X Read Buffer T to 10 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

1. **Addition of Diluent 2:** Dispense 25 μL of Diluent 2 into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
2. **Addition of the Sample or Calibrator:** Dispense 25 μL of sample or Calibrator into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
3. **Wash and Addition of the Detection Antibody**
Solution: Wash the plate 3X with PBS-T. Dispense 25 μL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3X with PBS-T. Add 150 μL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

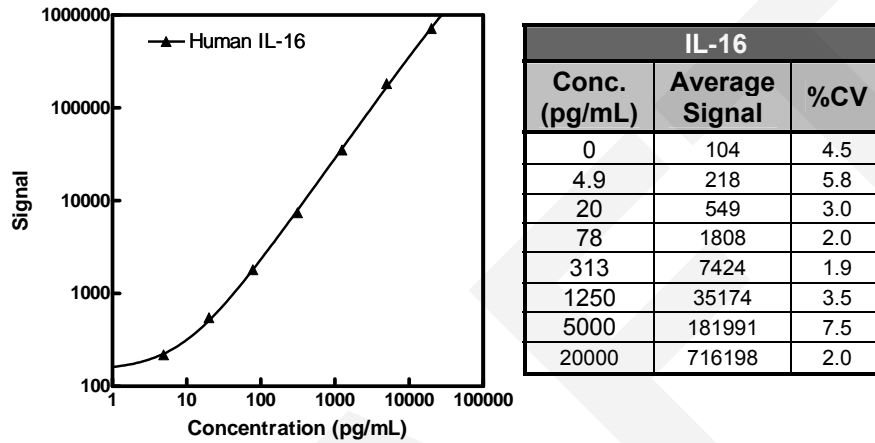
analysis of results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench[®] analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. The value below represents the average LLOD over multiple kit lots.

IL-16	
LLOD (pg/mL)	1.8

