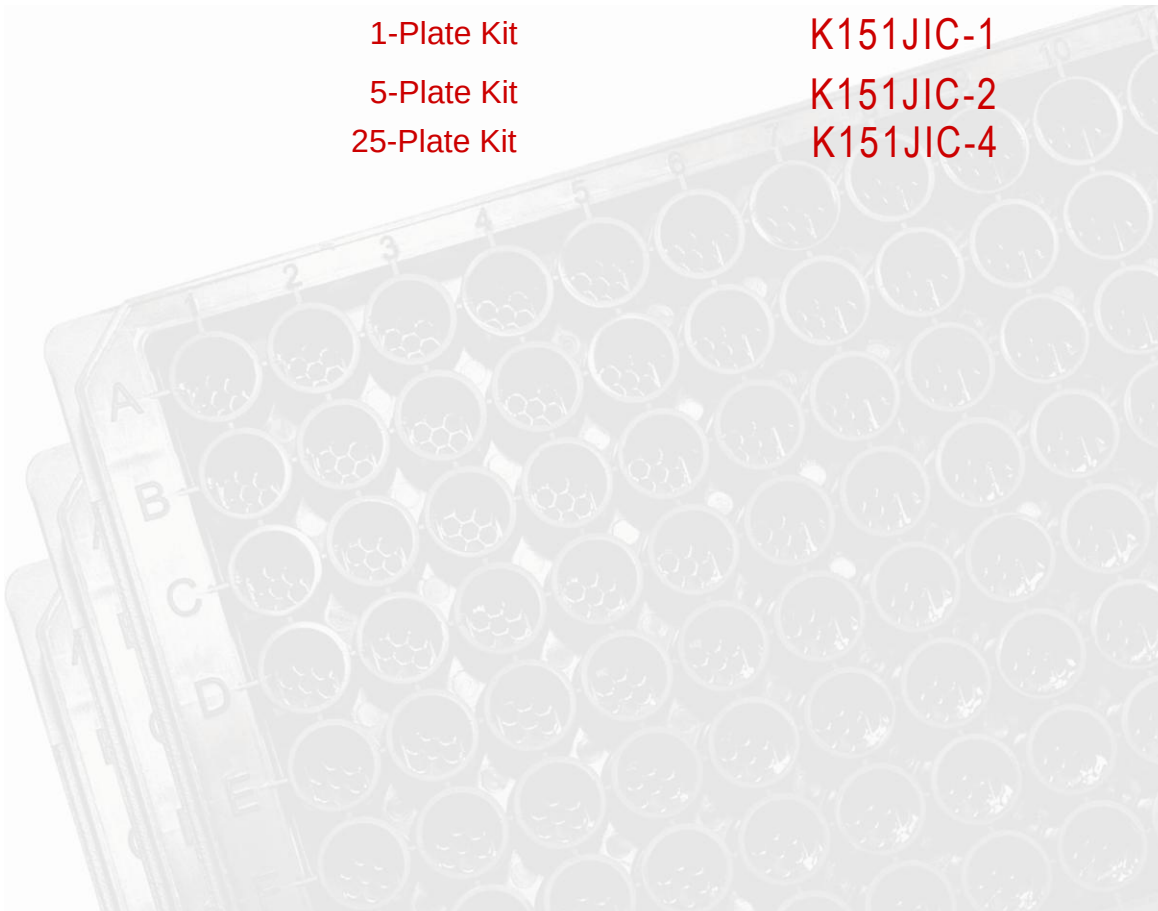
Meso Scale Discovery®

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



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.

Table of Contents

table of contents

Ordering Information

Ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

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-2Rα 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-1β, 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.

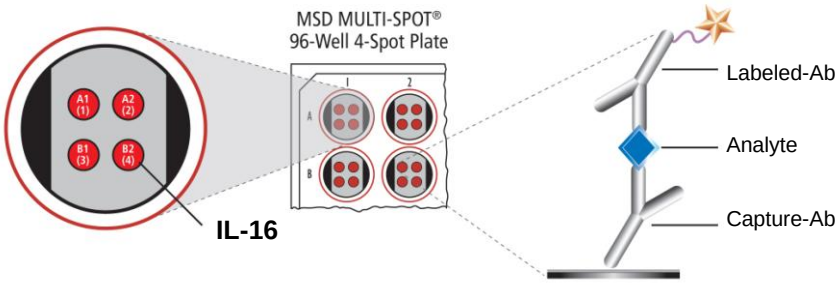


Figure 1. *Spot diagram showing placement of analyte capture antibody on Cytokine Panel 14 Plate. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability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

III 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 µL 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 µL of the Human IL-16 stock Calibrator to 990 µL Diluent 2.
2) Prepare the highest Calibrator point (STD-01) by transferring 50 µL of the Human IL-16 diluted stock Calibrator to 150 µL 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 µL 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

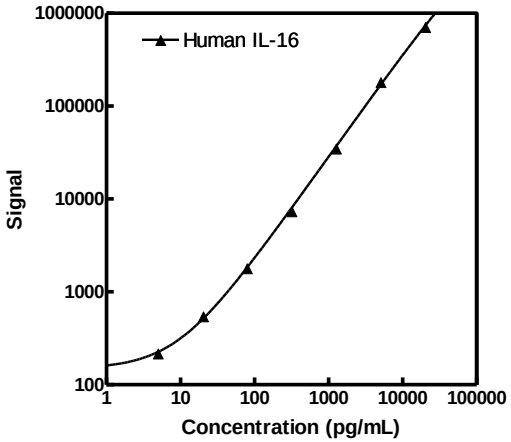
analysis of results

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.



IL-16		
Conc. (pg/mL)	**Average Signal**	**%CV**
0	104	4.5
4.9	218	5.8
20	549	3.0
78	1808	2.0
313	7424	1.9
1250	35174	3.5
5000	181991	7.5
20000	716198	2.0

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

XI Spike Recovery

spike recovery

Human serum and EDTA plasma pooled samples were spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates. An average of two serum and three EDTA plasma are shown here. Results of spike-recovery may vary based on the individual samples.
% Recovery = measured / expected x 100

Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	265	3.0	
	45	252	3.3	81
	377	586	4.2	91
	4037	4548	2.7	106
EDTA Plasma	0	239	3.2	
	45	232	4.1	82
	377	552	4.7	90
	4037	4403	7.0	103

XII Linearity

linearity

Three pools each of human serum and EDTA plasma were evaluated; a representative pool of each is shown below. The pooled samples were spiked with Calibrator and then diluted with Diluent 2. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = (measured x dilution factor) / expected x 100

Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	1	1612	2.3	
	2	1672	3.8	104
	4	1568	4.1	94
	8	1398	5.1	89
EDTA Plasma	1	1542	5.1	
	2	1447	7.6	94
	4	1343	6.8	93
	8	1174	3.8	87

XIII Samples

samples

Eight normal human samples were measured for each of the following sample types: serum, EDTA plasma, and heparin plasma.

		IL-16 (pg/mL)
Serum	Min	143
	Max	431
	Median	216
EDTA Plasma	Min	868
	Max	3106
	Median	1551
Heparin Plasma	Min	545
	Max	2790
	Median	1021

XIV References

references

1. Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. J Allergy Clin Immunol. 1993 Sep;92(3):412-24.
2. 1999 Feb 1;162(3):1287-93.Processing and release of IL-16 from CD4+ but not CD8+ T cells is activation dependent. J Immunol. 1999 Feb 1;162(3):1287-93.
3. Bannert N, Avots A, Baier M, Serfling E, Kurth R. GA-binding protein factors, in concert with the coactivator CREB binding protein/p300, control the induction of the interleukin 16 promoter in T lymphocytes. Proc Natl Acad Sci U S A. 1999 Feb 16;96(4):1541-6.
4. Laberge S, Pinsonneault S, Ernst P, Olivenstein R, Ghaffar O, Center DM, Hamid Q. Phenotype of IL-16-producing cells in bronchial mucosa: evidence for the human eosinophil and mast cell as cellular sources of IL-16 in asthma. Int Arch Allergy Immunol. 1999 Jun;119(2):120-5.
5. Franz JK, Kolb SA, Hummel KM, Lahrtz F, Neidhart M, Aicher WK, Pap T, Gay RE, Fontana A, Gay S. Interleukin-16, produced by synovial fibroblasts, mediates chemoattraction for CD4+ T lymphocytes in rheumatoid arthritis. Eur J Immunol. 1998 Sep;28(9):2661-71.
6. Kaser A, Dunzendorfer S, Offner FA, Ryan T, Schwabegger A, Cruikshank WW, Wiedermann CJ, Tilg H. A role for IL-16 in the cross-talk between dendritic cells and T cells. J Immunol. 1999 Sep 15;163(6):3232-8.
7. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, Kornfeld H. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem. 1998 Jan 9;273(2):1144-9.
8. Center DM, Kornfeld H, Cruikshank WW. Interleukin 16 and its function as a CD4 ligand. Immunol Today. 1996 Oct;17(10):476-81.
9. Cruikshank W, Center DM. Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). J Immunol. 1982 Jun;128(6):2569-74.
10. Parada NA, Center DM, Kornfeld H, Rodriguez WL, Cook J, Vallen M, Cruikshank WW. Synergistic activation of CD4+ T cells by IL-16 and IL-2. J Immunol. 1998 Mar 1;160(5):2115-20.
11. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol. 2000;67:757–66.
12. Cruikshank WW, Berman JS, Theodore AC, Bernado J, Center DM. Lymphokine activation of $T4^{+}$ lymphocytes and monocytes. J Immunol. 1987;138:3817–23.

13. Szabo P, Kesheng Z, Kirman I, et al. Maturation of B cell precursors is impaired in thymic-deprived nude and old mice. J Immunol. 1998;161:2248–53.
14. Mathy NL, Bannert N, Norley SG, Kurth R. Cutting edge: CD4 is not required for the functional activity of IL-16. J Immunol. 2000;164:4429–32.
15. Laberge S, Ghaffar O, Boguniewicz M, Center DM, Leung DY, Hamid Q. Association of increased CD4+ T-cell infiltration with increased IL-16 gene expression in atopic dermatitis. J Allergy Clin Immunol. 1998 Oct;102(4 Pt 1):645-50.
16. Schluesener HJ, Seid K, Kretzschmar J, Meyermann R. Leukocyte chemotactic factor, a natural ligand to CD4, is expressed by lymphocytes and microglial cells of the MS plaque. J Neurosci Res. 1996 Jun 15;44(6):606-11.
17. Lee S, Kaneko H, Sekigawa I, Tokano Y, Takasaki Y, Hashimoto H. Circulating interleukin-16 in systemic lupus erythematosus. Br J Rheumatol. 1998 Dec;37(12):1334-7.
18. Klimiuk PA, Goronzy JJ, Weyand CM. IL-16 as an anti-inflammatory cytokine in rheumatoid synovitis. J Immunol. 1999 Apr 1;162(7):4293-9
19. Seegert D, Rosenstiel P, Pfahler H, Pfefferkorn P, Nikolaus S, Schreiber S. Increased expression of IL-16 in inflammatory bowel disease. Gut. 2001 Mar;48(3):326-32
20. Keates AC, Castagliuolo I, Cruickshank WW, Qiu B, Arseneau KO, Brazer W, Kelly CP. Interleukin 16 is up-regulated in Crohn's disease and participates in TNBS colitis in mice. Gastroenteroloy. 2000 Oct;119(4):972-82.
21. Amiel C, Darcissac E, Truong MJ, Dewulf J, Loyens M, Mouton Y, Capron A, Bahr GM. Interleukin-16 (IL-16) inhibits human immunodeficiency virus replication in cells from infected subjects, and serum IL-16 levels drop with disease progression. J Infect Dis. 1999 Jan;179(1):83-91
22. Baier M, Kurth R. Fighting HIV-1 with IL-16. Nat Med. 1997 Jun;3(6):605-6.

Summary Protocol

MSD 96-well MULTI-ARRAY Human IL-16 Assay: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Human IL-16 Assay.

Step 1 : Sample and Reagent Preparation

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

Samples may not require dilution prior to use in this assay.

Prepare calibrator solutions and standard curve.

Use the 2 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 2.

Note: *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X in 3.0 mL of Diluent 3 (per plate).

Prepare 20 mL of 2X Read Buffer T by diluting 4X MSD Read Buffer T with deionized water.

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 2

Dispense 25 µL/well Diluent 2.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

Step 3: Add Sample or Calibrator

Dispense 25 µL/well Calibrator or sample.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 4: Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

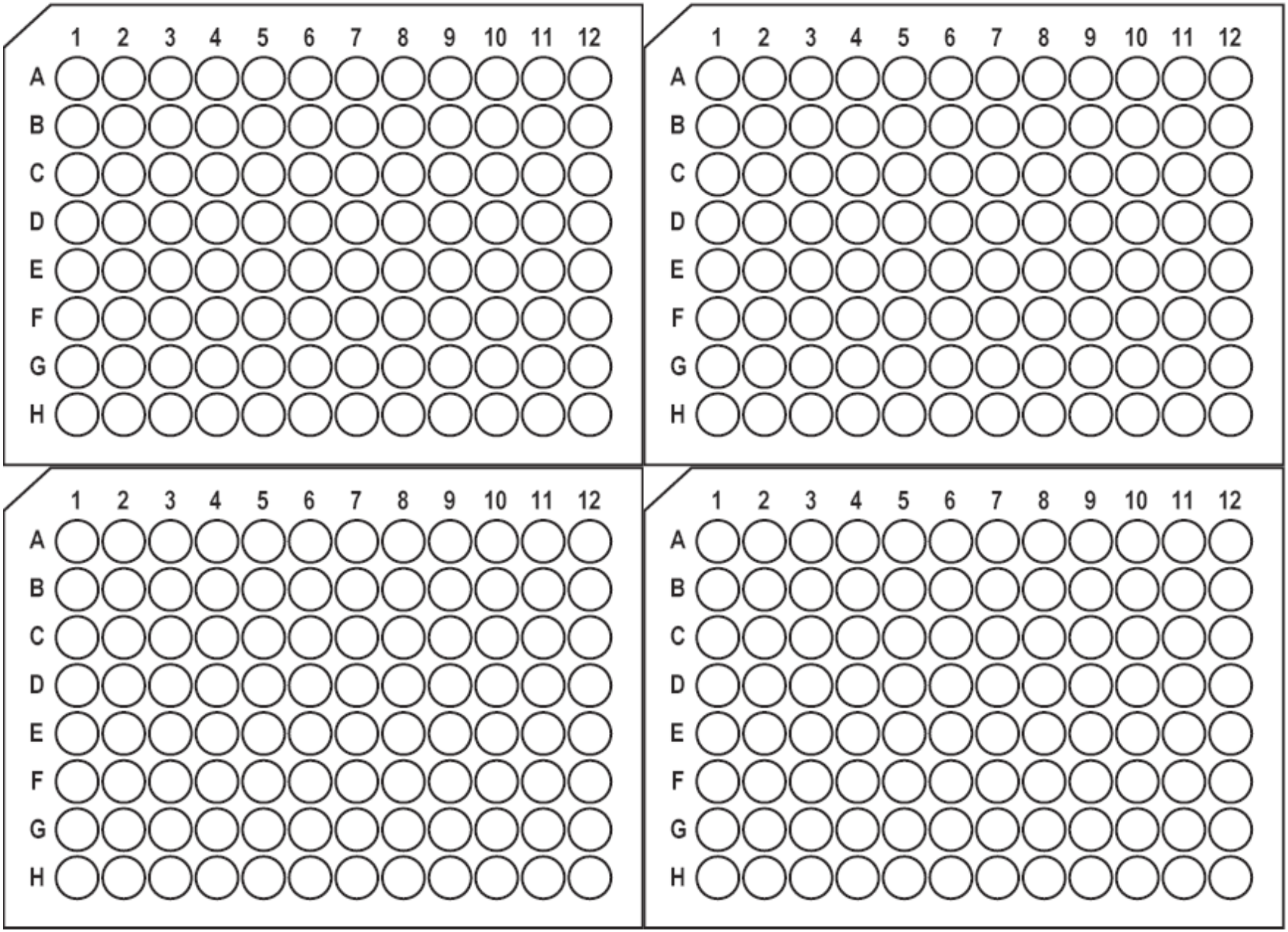
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 5: Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H