arigoPLEX® Human Inflammatory Cytokine Multiplex ELISA Kit (IL1 alpha, IL1 beta, IL6, IL8, GM-CSF, IFN-gamma, MCP1 and TNF-alpha)

Multiplex Enzyme Immunoassay for the semi-quantification of IL1 alpha, IL1 beta, IL6, IL8, GM-CSF, IFN-gamma, MCP1 and TNF-alpha in serum, plasma and cell culture supernatant.

Catalog number: ARG83509

Package: 96 wells

Lot. 374266

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

A proinflammatory cytokine or more simply an inflammatory cytokine is a type of signaling molecule (a cytokine) that is excreted from immune cells like helper T cells (Th) and macrophages, and certain other cell types that promote inflammation. They include interleukin-1 (IL1), IL12, and IL18, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), and granulocyte-macrophage colony stimulating factor and play an important role in mediating the innate immune response. Inflammatory cytokines are predominantly produced by and involved in the upregulation of inflammatory reactions.

A proinflammatory cytokine or an inflammatory cytokine is a type of cytokine (signaling molecule) that is excreted from immune cells and certain other cell types that promotes inflammation. Inflammatory cytokines are predominantly produced by helper T cells (Th) and macrophages and involved in the upregulation of inflammatory reactions. Therapies to treat inflammatory diseases include monoclonal antibodies that either neutralize inflammatory cytokines or their receptors.

Proinflammatory cytokines include interleukin-1 (IL1), IL12, and IL18, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), and granulocyte-macrophage colony stimulating factor.

Inflammatory cytokines play a role in initiating the inflammatory response and to regulate the host defense against pathogens mediating the innate immune response. Some inflammatory cytokines have additional roles such as acting as growth factors. Pro-inflammatory cytokines such as IL1β, IL6, and TNF-α also trigger pathological pain. While IL1β is released by monocytes and macrophages, it is also present in nociceptive DRG neurons. IL6 plays a role in

neuronal reaction to an injury. TNF-α is a well-known proinflammatory cytokine present in neurons and the glia. TNF-α is often involved in different signaling pathways to regulate apoptosis in the cells. Excessive chronic production of inflammatory cytokines contribute to inflammatory diseases. That have been linked to different diseases, such as atherosclerosis and cancer. Dysregulation of proinflammatory cytokines have also been linked to depression and other neurological diseases. A balance between proinflammatory and anti-inflammatory cytokines is necessary to maintain health. Aging and exercise also play a role in the amount of inflammation from the release of proinflammatory cytokines. [From wikipedia]

PRINCIPLE OF THE ASSAY

This is a multiplex enzyme immunoassay for the semi-quantification of Pro-inflammatory Cytokines Interleukin-1 alpha (IL1 alpha), Interleukin-1 beta (IL1 beta), Interleukin-6 (IL6), Interleukin-8 (IL8), Interferon Gamma (IFN-gamma), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Monocyte Chemotactic and Activating Factor (MCP1), and Tumor Necrosis Factor alpha (TNF-alpha). This assay employs the semi-quantitative sandwich enzyme immunoassay technique. Monoclonal antibody specific for IL1 alpha, IL1 beta, IL6, IL8, GM-CSF, IFN-gamma, MCP1 and TNF-alpha has been pre-coated onto wells of microtiter plate. Standards or samples are pipetted into the wells and any cytokine present is bound by the immobilized antibody. Then a biotin-conjugated antibody mixture is added to each well and incubate. After washing away any unbound substances, an HRP-conjugated avidin is added to each well and incubate. A substrate solution (TMB) is added to the wells and color

develops in proportion to the amount of cytokine bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Cytokine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate [1]	8 X 12 strips	4°C
Standards Mixture [2]	3 vial (lyophilized)	4°C
100X Antibody Conjugate Mixture	120 µL	≤ -20°C
1000X HRP-Streptavidin concentrate	15 µL	4°C
Standard / Sample Diluent Buffer	30 mL	4°C
Antibody Diluent Buffer	35 ml	4°C
10X Wash Buffer	50 mL	4°C
TMB substrate	12 mL (ready to use)	4°C (protect from light)
STOP solution	12 mL (ready to use)	4°C
Plate sealer	3 strips	RT

Note:

1. The Antibody Coated microplate contains twelve 8-well ELISA strips. Each of the eight wells has been coated with a different antibody specific to one of the 8 cytokines as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α	IL-1α
B	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
C	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
D	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8
E	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
F	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ
G	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1	MCP1
H	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TNF-α

2. Standards Mixture each vial contains a buffered protein base and four cytokines at different amount: IL1 alpha: 15.6 - 1000 pg/ml, IL1 beta: 7.8 - 500 pg/ml, IL6: 15.6 - 1000 pg/ml, IL8: 23.4 - 1500 pg/ml, GM-CSF: 7.8 - 500 pg/ml, IFN-gamma: 7.8 - 500 pg/ml, MCP1: 15.6 - 1000 pg/ml, TNF alpha: 15.6 - 1000 pg/ml.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store 100X Antibody Conjugate at ≤ -20°C. Store other component at 2-8°C at all times.
- The reconstituted standard stock should be aliquoted and stored at -80°C.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in

the frame provided.

- All reagents must be mixed without foaming and briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the mixed TMB Substrate. Do not expose the mixed TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- All reagents must be mixed without foaming before use.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

c) Avoid disturbing the white buffy layer when collection serum / plasma sample.

d) To obtain the data of each cytokine, at least 0.4 mL of the sample is needed to complete one run of the assay. It is recommended to have a larger volume available in case that the experiments have to be repeated.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C. Mix well before use.
- 1X Antibody Conjugate Mixture: It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute 100X Antibody Conjugate Mixture concentrate into Standard / Sample Diluent Buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X Antibody Conjugate Mixture concentrate + 990 µl of Diluent Buffer)
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute

1000X HRP-Streptavidin concentrate solution into Standard / Sample Diluent Buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 1 µl of 1000X HRP-Streptavidin concentrate solution + 999 µl of Diluent Buffer)

- Sample: Before assay, serum and plasma are recommended to dilute with equal volume of Standard/Sample Diluent Buffer. (eg. Premix 250 µL sample with 250 µL Standard/Sample Diluent Buffer.)
- Standards Mixture:

A. Add 1 mL of Standard / Sample Diluent Buffer to reconstitute the lyophilized Standard Mixture to obtain the high concentration stock of 8 cytokines at different concentrations (see table below). Brief vortex and allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The reconstituted Standard Mixture high concentration stock could be stored at -80°C for up to 30 days.

B. For semi-quantitative assay, use the above high concentration Standards Mixture and a 32-fold diluted low concentration Standards Mixture to test together with up to 22 test samples. If more accurate results are required, a two-fold serial dilution with Dilution Buffer can generate a more accurate standard curve. However, the number of test samples will be reduced.

The concentrations of the 8 cytokines in different dilutions of the Standards Mixture are listed as below:

Cytokine (pg/ml)	Stock	1:2	1:4	1:8	1:16	1:32	1:64
A: IL1a	1000	500	250	125	62.5	31.25	15.63
B:IL1b	500	250	125	62.5	31.25	15.63	7.81
C:IL6	1000	500	250	125	62.5	31.25	15.63
D:IL8	1500	750	375	187.5	93.75	46.88	23.44
E:GM-CSF	500	250	125	62.5	31.25	15.63	7.81
F:IFN-g	500	250	125	62.5	31.25	15.63	7.81
G:MCP1	1000	500	250	125	62.5	31.25	15.63
H:TNF-a	100	50	25	12.5	6.25	3.13	1.56

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use.

1. Add **100 µL** of the **Standards Mixture** or **diluted samples** to the Antibody Coated microplate.

   Note: To obtain the approximate concentrations of 8 cytokines on 10 test samples, the low concentration standard mixture (**S1, 1:32 from high concentration mixture**), the high concentration Standards Mixture (**S2, stock**) and test samples (T1 to T10) can be added as the scheme as below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
B	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
C	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
D	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
E	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
F	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
G	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
H	S1	S2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10

2. Cover the plate and incubate for **2 hours** at **room temperature**.
3. Aspirate each well and wash, repeating the process 4 times for a **total 5 time washes**. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Antibody Conjugate Mixture** to each wells.
5. Cover the plate and Incubate for **1 hour** at **room temperature**.
6. Aspirate each well and **wash as step 3.**
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well. Cover the plate and incubate for **1 hour** at room temperature.
8. Aspirate each well and **wash as step 3**.
9. Add **100 µL** of **TMB Substrate** to each well. Cover and incubate for **10-20 minutes** at **room temperature** in the dark.
10. Immediately Add **100 µL** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
11. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 30 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following data shows the typical standard curve a run of this multiplex ELISA with multiple dilutions using standard diluent I. It is for demonstration purpose only and cannot be used to replace standard curve for testing. Each investigators have to assay standards along with test samples.

Detection Range

IL1 alpha: 15.6- 1000 pg/ml

IL1 beta: 7.8- 500 pg/ml

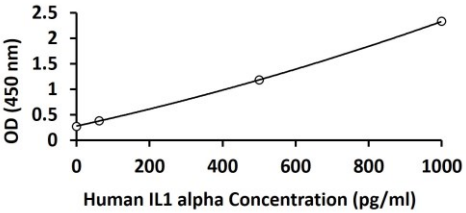
IL6: 15.6- 1000 pg/ml

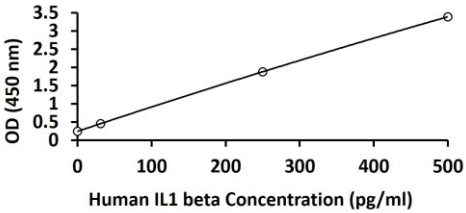
IL8: 23.4- 1500 pg/ml

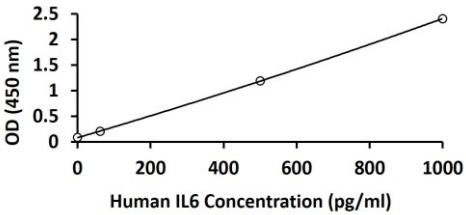
GM-CSF: 7.8- 500 pg/ml

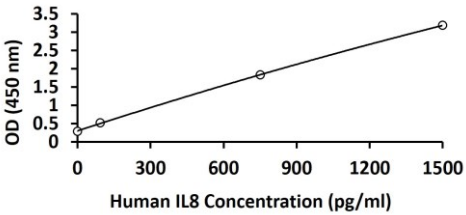
IFN-gamma: 7.8- 500 pg/ml

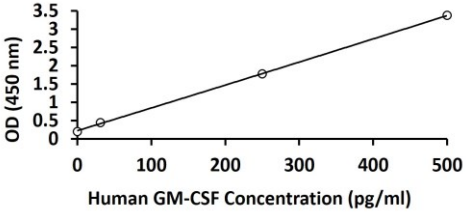
MCP1: 15.6- 1000 pg/ml

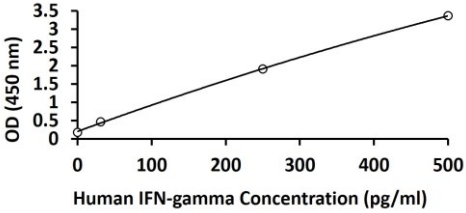

OD (450 nm)
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
Human IL1 alpha Concentration (pg/ml)

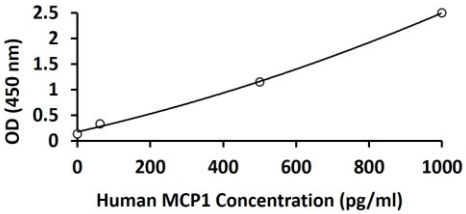

OD (450 nm)
3.5
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Human IL1 beta Concentration (pg/ml)

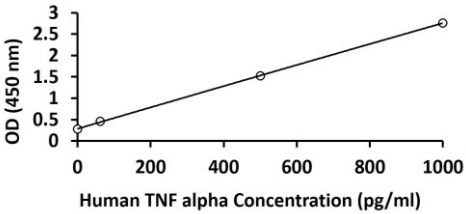

OD (450 nm)
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
Human IL6 Concentration (pg/ml)


OD (450 nm)
3.5
3
2.5
2
1.5
1
0.5
0
0
300
600
900
1200
1500
Human IL8 Concentration (pg/ml)


OD (450 nm)
3.5
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Human GM-CSF Concentration (pg/ml)


OD (450 nm)
3.5
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Human IFN-gamma Concentration (pg/ml)


OD (450 nm)
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
Human MCP1 Concentration (pg/ml)


OD (450 nm)
3
2.5
2
1.5
1
0.5
0
0
200
400
600
800
1000
Human TNF alpha Concentration (pg/ml)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. The 4 curves for 4 targets can be generated from OD readings of high concentration standard and low concentration standard mixture. The standard curves might not be perfectly straight. To obtain more accurate results, more dilution points can be used when generating standard curves.
3. 4 Parameter Logistics is the preferred method for the result calculation. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. Serum/plasma sample has multiplied the dilution factor (Dilution factor =2). If the samples have been further diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.