arigoPLEX® Human M1/M2/MDSC Cytokine Multiplex ELISA Kit (GM-CSF, IFN gamma, IL4, IL6, IL10, IL12, MCP1, TNF alpha)

arigoPLEX® Human M1/M2/MDSC Cytokine Multiplex ELISA Kit (GM-CSF, IFN gamma, IL4, IL6, IL10, IL12, MCP-1, TNF alpha) is an Enzyme Immunoassay kit for the quantification of GM-CSF, IFN gamma, IL4, IL6, IL10, IL12, MCP1 and TNF alpha in serum, plasma and cell culture supernatant.

Catalog number: ARG83474

Package: 96 wells

Lot. 374175

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

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INTRODUCTION

GM-CSF is a cytokine that stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes. [UniProt]

IFN gamma is produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons. [UniProt]

IL4 participates in at least several B-cell activation processes as well as of other cell types. It is a costimulator of DNA-synthesis. It induces the expression of class II MHC molecules on resting B-cells. It enhances both secretion and cell surface expression of IgE and IgG1. It also regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes. [UniProt]

IL6 is a cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. Required for the generation of T(H)17 cells. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation. [UniProt]

IL10 inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells. [UniProt]

IL-12 is primarily produced by professional antigen-presenting cells (APCs) such as B-cells and dendritic cells (DCs) as well as macrophages and granulocytes and regulates T-cell and natural killer-cell responses, induces the production of interferon-gamma (IFN-gamma), favors the differentiation of T-helper 1 (Th1) cells and is an important link between innate resistance and adaptive immunity. [UniProt]

MCP-1 is a chemotactic factor that attracts monocytes and basophils but not neutrophils or eosinophils. Augments monocyte anti-tumor activity. Has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, like psoriasis, rheumatoid arthritis or atherosclerosis. May be involved in the recruitment of monocytes into the arterial wall during the disease process of atherosclerosis. [UniProt]

TNF alpha is a cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation. Impairs regulatory T-cells (Treg) function in individuals with rheumatoid arthritis via FOXP3 dephosphorylation. Upregulates the expression of protein phosphatase 1 (PP1), which dephosphorylates the key 'Ser-418' residue of FOXP3, thereby inactivating FOXP3 and rendering Treg cells functionally defective (PubMed:23396208). The TNF intracellular domain (ICD) form induces IL12 production in dendritic cells. [UniProt]

PRINCIPLE OF THE ASSAY

This is a multiplex enzyme immunoassay for the quantification of M1/M2/MDSC macrophagy Cytokines GM-CSF, IFN gamma, IL4, IL6, IL10, IL12, MCP1, TNF alpha. This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibody specific for GM-CSF, IFN gamma, IL4, IL6, IL10, IL12, MCP1 and TNF alpha has been pre-coated onto a 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	8 X 12 strips	4°C
Standards Mixture	3 vials	4°C
100X Antibody Conjugate Mixture	120 ul	≤ -20°C
1000X HRP-Streptavidin solution	15 ul	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	4°C (protect from light)
STOP solution	12 ml	4°C
Plate sealer	3 strips	Room temperature

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	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF
B	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ	IFN-γ
C	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4
D	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
E	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10	IL-10
F	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12	IL-12
G	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
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: GM-CSF: 15.6-500 pg/ml; IFN gamma: 15.6-500 pg/ml; IL4: 15.6-500 pg/ml; IL6: 31.25-1000 pg/ml; IL10: 15.6-500

pg/ml; IL12: 31.25-1000 pg/ml; MCP1: 31.25-1000 pg/ml; TNF alpha: 31.25-1000 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. 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:

Cytokines (pg / mL)	High Conc. Standard Stock	1:2	1:4	1:8	1:16	1:32 (Low Conc. Standard Mixture)	1:64
GM-CSF	500	250	125	62.5	31.25	15.63	7.81
IFN gamma	500	250	125	62.5	31.25	15.63	7.81
IL4	500	250	125	62.5	31.25	15.63	7.81
IL6	1000	500	250	125	62.5	31.25	15.63
IL10	500	250	125	62.5	31.25	15.63	7.81
IL12	1000	500	250	125	62.5	31.25	15.63
MCP1	1000	500	250	125	62.5	31.25	15.63
TNF alpha	1000	500	250	125	62.5	31.25	15.63

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.

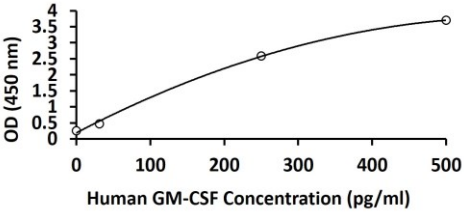
CALCULATION OF RESULTS

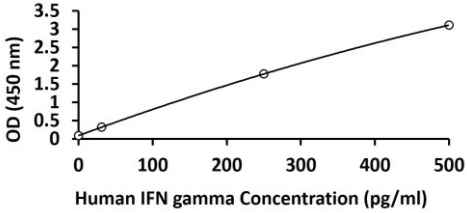
1. Calculate the average absorbance values for each set of standards and samples.
2. The 8 curves for 8 targets can be generated from OD readings of high concentration standard and low concentration standard mixture. The standard curves might not be perfectly straight. To obtain more accurate results, 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)

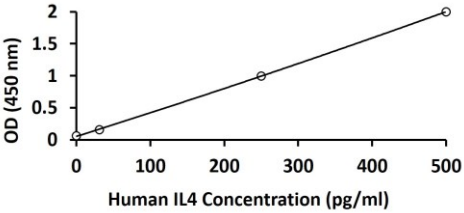
5. Serum/plasma sample has multiplied the dilution factor (Dilution factor =2). If the samples have been further diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.

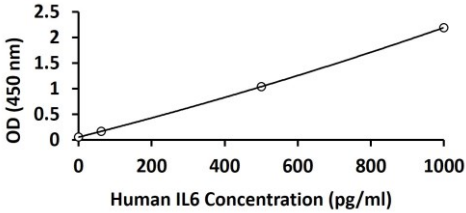
EXAMPLE OF TYPICAL STANDARD CURVE

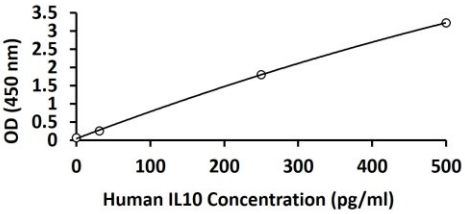
The following data shows the typical standard curve a run of this multiplex ELISA with multiple dilutions using standard diluent I. It is for demonstration purpose only and cannot be used to replace standard curve for testing. Each investigators have to assay standards along with test samples.

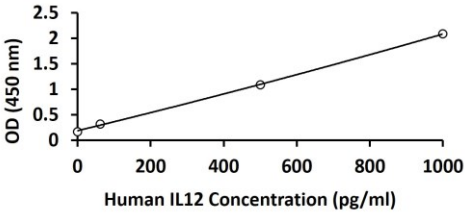


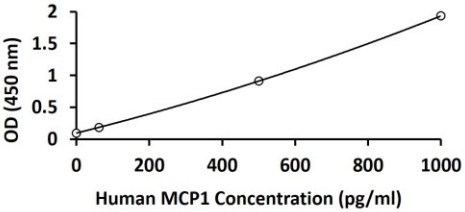


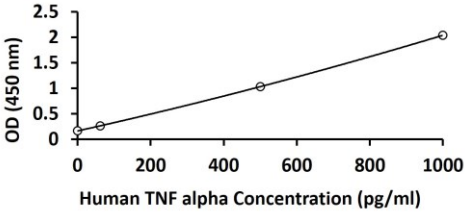












Detection Range

GM-CSF: 15.6-500 pg/ml

IFN gamma: 15.6-500 pg/ml

IL4: 15.6-500 pg/ml

IL6: 31.25-1000 pg/ml

IL10: 15.6-500 pg/ml

IL12: 31.25-1000 pg/ml

MCP1: 31.25-1000 pg/ml

TNF alpha: 31.25-1000 pg/ml