arigoPLEX® Human Gamma Delta T cell multiplex ELISA Kit (IL8, IL17, IFN-gamma, Granzyme b)

arigoPLEX® Human Gamma Delta T cell multiplex ELISA Kit (IL8, IL17, IFN-gamma, Granzyme b) is an Enzyme Immunoassay kit for the quantification of Human Gamma Delta T cell (IL8, IL17, IFN gamma, Granzyme B) in serum, plasma and cell culture supernatants.

Catalog number: ARG83110

Package: 96 wells

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INTRODUCTION

IL8: The protein encoded by this gene is a member of the CXC chemokine family and is a major mediator of the inflammatory response. The encoded protein is commonly referred to as interleukin-8 (IL-8). IL-8 is secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. It functions as a chemotactic factor by guiding the neutrophils to the site of infection. Bacterial and viral products rapidly induce IL-8 expression. IL-8 also participates with other cytokines in the proinflammatory signaling cascade and plays a role in systemic inflammatory response syndrome (SIRS). This gene is believed to play a role in the pathogenesis of the lower respiratory tract infection bronchiolitis, a common respiratory tract disease caused by the respiratory syncytial virus (RSV). The overproduction of this proinflammatory protein is thought to cause the lung inflammation associated with csytic fibrosis. This proinflammatory protein is also suspected of playing a role in coronary artery disease and endothelial dysfunction. This protein is also secreted by tumor cells and promotes tumor migration, invasion, angiogenesis and metastasis. This chemokine is also a potent angiogenic factor. The binding of IL-8 to one of its receptors (IL-8RB/CXCR2) increases the permeability of blood vessels and increasing levels of IL-8 are positively correlated with increased severity of multiple disease outcomes (eg, sepsis). This gene and other members of the CXC chemokine gene family form a gene cluster in a region of chromosome 4q.

IL17: This gene is a member of the IL-17 receptor family which includes five members (IL-17RA-E) and the encoded protein is a proinflammatory cytokine

produced by activated T cells. IL-17A-mediated downstream pathways induce the production of inflammatory molecules, chemokines, antimicrobial peptides, and remodeling proteins. The encoded protein elicits crucial impacts on host defense, cell trafficking, immune modulation, and tissue repair, with a key role in the induction of innate immune defenses. This cytokine stimulates non-hematopoietic cells and promotes chemokine production thereby attracting myeloid cells to inflammatory sites. This cytokine also regulates the activities of NF-kappaB and mitogen-activated protein kinases and can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). IL-17A plays a pivotal role in various infectious diseases, inflammatory and autoimmune disorders, and cancer. High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis. The lung damage induced by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is to a large extent, a result of the inflammatory response promoted by cytokines such as IL17A.

IFN gamma: This gene encodes a soluble cytokine that is a member of the type II interferon class. The encoded protein is secreted by cells of both the innate and adaptive immune systems. The active protein is a homodimer that binds to the interferon gamma receptor which triggers a cellular response to viral and microbial infections. Mutations in this gene are associated with an increased susceptibility to viral, bacterial and parasitic infections and to several autoimmune diseases.

Granzyme B: This gene encodes a member of the granzyme subfamily of proteins, part of the peptidase S1 family of serine proteases. The encoded preproprotein is secreted by natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) and proteolytically processed to generate the active protease, which induces target cell apoptosis. This protein also processes cytokines and degrades extracellular matrix proteins, and these roles are implicated in chronic inflammation and wound healing. Expression of this gene may be elevated in human patients with cardiac fibrosis.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. The antibodies specific to IL8, IL17, IFN gamma and Granzyme B have been pre-coated onto a microtiter plate. Standards Mixture and samples are pipetted into the wells and any target present is bound by the immobilized specific antibodies. After incubation, added Mixed Antibody-Conjugate to each well and incubate. After washing away any unbound substances, the HRP Conjugate is added into the wells and incubated. The wells are thoroughly washed to remove all unbound HRP Conjugate. A TMB substrate is added to the wells and color develops in proportion to the amount of target in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of O.D. 450 nm. The concentration of Human T cell in the samples is then determined by comparing the O.D. 450 nm of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store other components at 2-8°C at all times. 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 Solution	15 µL	4°C
Diluent Buffer	50 mL (ready to use)	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	4 adhesive 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 4 targets as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8
B	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17
C	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma
D	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B
E	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8	IL8
F	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17	IL17
G	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma	IFN gamma
H	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B	Granzyme B

2. Standards Mixture each vial contains a buffered protein base and four targets at different amount: IL-8: 1500 pg; IL-17: 500 pg; IFN gamma: 500 pg; Granzyme B: 1000 pg.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (optional)

TECHNICAL NOTES 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 targets, at least **0.2 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 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 Solution** into Diluent Buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 1 µl of 1000X HRP-Streptavidin Solution + 999 µl of Diluent Buffer)

- **Sample:** Before assay, serum and plasma are recommended to dilute with equal volume of Diluent Buffer. (eg. Premix 250 µL sample with 250 µL Diluent Buffer.)
- **Standards Mixture**:
  A. Add **1 mL** of **Diluent Buffer** to reconstitute the lyophilized Standard Mixture to obtain the high concentration stock of 4 targets 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 4 targets in different dilutions of the Standards Mixture are listed as below:

targets (pg / mL)	High Conc. Standard Stock	1:2	1:4	1:8	1:16	1:32 (Low Conc. Standard)	1:64
IL8	1500	750	375	187.5	93.75	46.88	23.44
IL17	500	250	125	62.5	31.25	15.63	7.81
IFN gamma	500	250	125	62.5	31.25	15.63	7.81
Granzyme B	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 4 targets on 22 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 T22) can be added as the scheme as below:

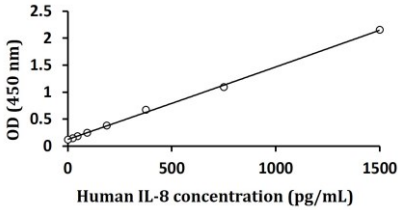
	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	T1	T3	T5	T7	T9	T11	T13	T15	T17	T19	T21
B	S1	T1	T3	T5	T7	T9	T11	T13	T15	T17	T19	T21
C	S1	T1	T3	T5	T7	T9	T11	T13	T15	T17	T19	T21
D	S1	T1	T3	T5	T7	T9	T11	T13	T15	T17	T19	T21
E	S2	T2	T4	T6	T8	T10	T12	T14	T16	T18	T20	T22
F	S2	T2	T4	T6	T8	T10	T12	T14	T16	T18	T20	T22
G	S2	T2	T4	T6	T8	T10	T12	T14	T16	T18	T20	T22
H	S2	T2	T4	T6	T8	T10	T12	T14	T16	T18	T20	T22

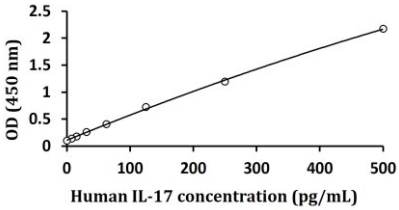
2. Cover the plate and incubate for **2 hours** at **room temperature**.
3. Aspirate each well and wash, repeating the process 4 times for a **total 5 time washes**. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of 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.**

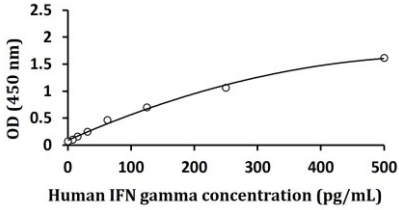
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well. Cover the plate and incubate for **1 hour** at **room temperature**.
8. Aspirate each well and **wash as step 3**.
9. Add **100 µL** of **TMB Substrate** to each well. Cover and incubate for **15-25 minutes** at **room temperature** 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.

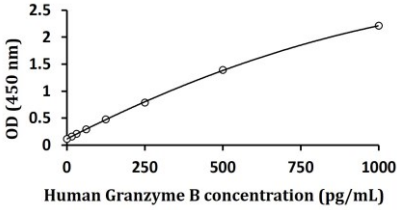
EXAMPLE OF TYPICAL STANDARD VALUES

The following table shows the OD readings of a run of this multiplex ELISA with serial diluted standards. It is for demonstration purpose only and cannot be used to replace the standard curve for testing. For each investigation, standards have to be assayed along with test samples and only the curve generated from the same test can be used.









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.