Human IL29 / IFN lambda ELISA Kit

Enzyme Immunoassay for the quantification of Human Interleukin-29(Interferon-λ) in serum, plasma, cell culture supernatants

Catalog number: ARG80169

Package: 96 wells

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

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INTRODUCTION

IL-29 is distantly related to type I interferons and the IL-10 family. Expression of IL-29 is induced by viral infection which interacts with a heterodimeric class II cytokine receptor that consists of interleukin 10 receptor beta (IL10RB) and interleukin 28 receptor alpha . IL-29 exhibits common features with type I IFNs such as antiviral activity, antiproliferative activity and in vivo antitumour activity. IL-29 acts similarly to IFNs, but is less effective generally and has activity in a more limited range of cell lines. IL-29 produced in response to viral infection, activates both monocytes and macrophages producing a restricted panel of cytokines and therefore is an important factor in activating innate immune responses at the site of viral infection. IFN-Lambda 1 antiviral and antiproliferative activity requires Interferon-Lambda 2 receptor tyrosine residues. Recombinant human IL-29 produced in E.Coli is a single, non-glycosylated, polypeptide chain containing 181 amino acids and having a molecular mass of?20 kDa.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IL29 / IFN lambda has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IL29 / IFN lambda present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for IL29 / IFN lambda is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any

unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IL29 / IFN lambda 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 IL29 / IFN lambda 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.

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
C2	Standard (Lyophilized)	3 X 0.4 ng/vial	4°C
C3	Standard diluent buffer	20 ml (ready to use)	4°C
C4	Antibody conjugate concentrate	1 vial (400 µl)	4°C
C5	Antibody diluent buffer	16 ml (ready to use)	4°C
C6	HRP-Streptavidin concentrate	1 vial (400 µl)	4°C (Protect from light)
C7	HRP-Streptavidin diluent buffer	16 ml (ready to use)	4°C
C8	20X Wash buffer	50 ml	4°C
C9	TMB substrate	12 ml (ready to use)	4°C (Protect from light)
C10	STOP solution	12 ml (ready to use)	4°C
C11	Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.

- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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.

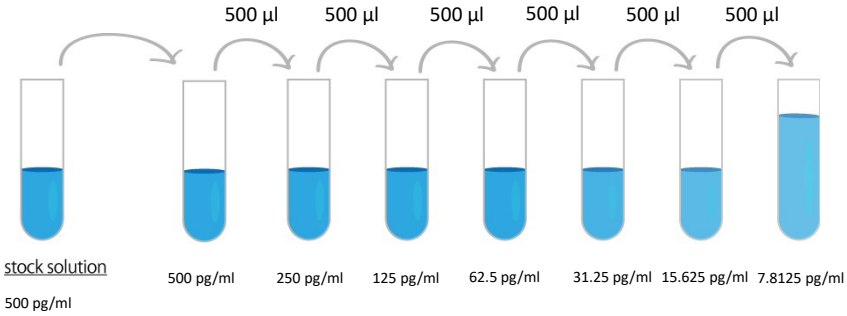
b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **20X** Wash buffer (#C8) into **distilled water** to yield 1X Wash buffer. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate**: 20 minutes before use, dilute **30X** antibody conjugate concentrate (#C4) into **antibody diluent buffer** (#C5) to yield 1X Detection antibody solution.
- **1X HRP-Streptavidin Solution**: 20 minutes before use, dilute **30X** HRP-Streptavidin concentrate solution (#C6) into **HRP-Streptavidin diluent buffer** (#C7) to yield 1X HRP-Streptavidin Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Sample**: If the initial assay found samples contain IL29 / IFN lambda higher than the highest standard, the samples can be diluted with Standard diluent buffer (#C3) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: Reconstitute the standard (#C2) with **800 µl** standard diluent buffer (#C3) to yield a stock concentration of **500 pg/ml.** Keep the buffer in the vail for at least **15 min at RT** to make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer

serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml, 7.8125 pg/ml**. DO NOT reuse the reconstituted standard.



Dilute IL29 / IFN lambda standard as according to the table below:

Standard	IL29 / IFN lambda Conc.	µl of Standard diluent	µl of standard
S7	500 pg/ml	0	1000 (500 pg/ml Stock)
S6	250 pg/ml	500	500 (S7)
S5	125 pg/ml	500	500 (S6)
S4	62.5 pg/ml	500	500 (S5)
S3	31.25 pg/ml	500	500 (S4)
S2	15.625 pg/ml	500	500 (S3)
S1	7.8125 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min before use.

Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of standards, samples and zero controls (standard diluent buffer) into wells, gently tap the plate to mix well. Incubate for **1.5 h at 37°C.**
3. Aspirate each well and wash, repeating the process four times for a total **five washes**. Wash by filling each well with 1× Wash Buffer (**350 µl**) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. 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** 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for **1 hour at 37°C**.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for **30 minutes at 37°C** in dark.
7. Aspirate each well and **wash as step 3**.
8. Add **100 µl** of TMB Reagent (#C9) to each well, gently tap the plate to mix well. Incubate for **15 minutes at 37°C** in dark.
9. Add **100 µl** of Stop Solution (#C10) to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at **450 nm immediately**. It is recommended read the absorbance within 3 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

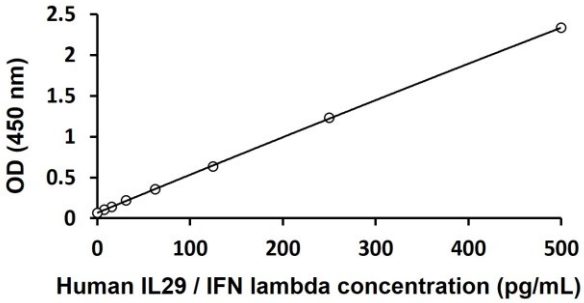
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. 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)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human IL29 / IFN lambda ranged from 7.8-500 pg/ml. The mean MDD was 4.3 pg/ml.

Specificity

This assay recognizes natural and recombinant Human IL29 / IFN lambda. No significant cross-reactivity or interference with the factors below was observed: Human IFN-αA/D, IFN-αA, IFN-αB2, IFN-αG, IFN-αI, IFN-β1a, IL-10 RβFc Chimera

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.