Human IL-29 (IFN lambda 1) ELISA Ready-SET-Go!®

Catalog Number: 88-7296
Also Known As: Interleukin-29
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: Human IL-29 (IFN lambda 1) ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7296
Sensitivity: 8 pg/mL
Standard Curve Range: 8 - 1000 pg/mL

Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to Vial
Use By: Refer to Vial
Caution, contains Azide

Description
IFN-λs are a novel family of interferons which mediate the induction of anti-viral protection in a wide variety of cells. The three members of the IFN-λ family are λ1, λ2, and λ3, also known as IL-29, IL-28A, and IL-28B, respectively. IFN-λs share with type I IFNs an intracellular signaling pathway that drives the expression of a common set of IFN-stimulated genes. IFN-lambdas induce multiple biological activities, including the upregulation of class I MHC gene product expression to levels comparable to those induced by IFN-αs. IL-28 and IL-29 are tested for anti-viral activity by challenging the human hepatocellular carcinoma cell line HepG2 with infection by EMC (following pretreatment of the cells with cytokine).

Consistent with a role in anti-viral protection, the mRNA expression of IFN-lambdas is detectable in cells infected with various viruses. Moreover, monocyte-derived dendritic cells (important producers of IFN-α) express IFN-λ1 mRNA in response to treatment with dsRNA. TLR3 and TLR4 ligands induce IFN-α, IFN-β, IL-28, and IL-29 gene expression in macrophages; this is dependent upon IFN-α.

IFN-lambdas mediate their anti-viral protection through a class II cytokine receptor complex distinct from that of type I IFNs. This is comprised of two essential receptor proteins, CRF2-12/IFN-λR1, which is unique to IFN-lambdas, and CFR2-4/IL-10R2, which is shared with IL-10, IL-22, and IL-26 receptors. Whereas, the two chains of the type I IFN receptor (IFN-AR1 and IFN-AR2) and IL-10R2 are ubiquitously expressed, IFN-λR1 expression is limited and cell-type dependent. IFN-λR1 is not expressed by monocytes, but is up-regulated during GM-CSF/IL-4 induced differentiation of DCs from human monocytes, yielding iDCs which are fully responsive to IFN-λ.

The IFN-λs, IL-28 and IL-29, have recently been reported to prime dendritic cells to induce proliferation of Foxp3-bearing regulatory T cells. IFN-λ-matured DCs express high levels of class I and II MHC gene products, but low levels of costimulatory molecules, and are able to specifically induce IL-2-dependent proliferation of CD4+CD25+FOXP3+ T cell population with contact dependent suppressive activity on T cells.

This Human IL-29/IFN-λ Ready-SET-Go! ELISA Set contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of human IL-29 protein levels from samples including serum, plasma, and supernatants from cell cultures. The assay demonstrates parallelism in measuring recombinant and native human IL-29 proteins with a standard curve range of 8.0 pg/ml to 1,000 pg/ml, and assay sensitivity below 8.0 pg/ml. The assay has been validated by specific detection of significant levels (e.g., >500 pg/ml) of native human IL-29 protein in supernatants from human GM-CSF - derived dendritic cells activated for 24 hrs with polyIC and CD40-His.

Components
Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant cytokine for generating standard curve and calibrating samples
ELISA/ELISPOT Coating Buffer Powder. This Ready-Set-Go! ELISA Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH20 and filter (0.22 uM)) or 10X PBS ELISA Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH20).
Assay Diluent. 5X concentrated
Detection enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Substrate Solution
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards
96 Well Plate. Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)

References
Kotenko, S., et al. 2003. IFN-lambdas mediate anti-viral protection through a distinct class II cytokine receptor complex. Nature Immunol. 4: 69-77.

Mennechet, F., et al. 2006. IFN-lambda-treated dendritic cells specifically induce proliferation of FOXP3-expressing suppressor T cells. Immunobiology. 107: 4417-4423.

Sheppard, P., et al. 2003. IL-28, IL-29, and their class II cytokine receptor IL-28R. Nature Immunol. 4: 63-68.

Related Products
34-8299 Human IL-29 (IFN lambda 1) Recombinant Protein Carrier-Free
34-8318 Human IFN alpha 1 Recombinant Protein Carrier-Free (Discontinued)
88-8999 Human Regulatory T Cell Staining Kit (PCH101)

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Enzyme Linked Immunosorbent Assay (ELISA)

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Protocol: ELISA Ready-Set-Go!

The following protocol is a general guideline for the Ready-SET-Go! Sets

Materials Provided

- Please refer to the Certificate of Analysis (C of A) for components

Other Materials Needed

- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M H_3PO_4 (recommended) or 2N H_2SO_4
- Pipettes
- Refrigerator & frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp®)
  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp (Cat. No. 44-2404) 96-well plates
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

Experimental Procedure

1. Coat Corning Costar 9018 (or Nunc Maxisorp®) ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with >250 µL/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µL/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Optional: Aspirate and wash at least once with Wash Buffer.
5. Using 1X Assay Diluent*, dilute standards as noted on the C of A to prepare the top concentration of the standard. Add 100 µL/well of top standard concentration to the appropriate

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wells. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points. Add 100 µL/well of your samples to the appropriate wells. Seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).

6. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
7. Add 100 µL/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
9. Add 100 µL/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes**.
11. Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µL of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

NOTES:

**** Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.***

*****The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.***

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Quick Guide: Standard Calibration

The following table indicates the protein standard contained in the Ready-SET-Go! is calibrated against NIBSC standards.

Table of Standard Calibration

Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-8	1	1.8	180	89/520
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIL-17A	1	0.9	9000	01/420
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532

* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)

ELISA Troubleshooting Guide

Problem	Possibility	Solution
A. High background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA being careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless. Replace
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat using correct dilutions
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA using the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA making no enzyme inhibitor is present in any buffers.

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	4. Coated capture antibody in Assay Diluent rather than Coating Buffer	4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody.
C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly, with a soak time.
	2. Incorrect dilutions of standard	2. Follow recommendations of standard preparation exactly as written on the C of A
	3. Insufficient incubation time	3. Repeat ELISA following the protocol carefully for each step
	4. Incorrect storage of reagents	4. Store reagents at the correct temperature asn indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards.
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see C of A. Edge effects can be avoided by moving samples and standards in from the edge of the plate.
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Reagents have expired	4. Order a new Ready-Set-Go ELISA.

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