pbl assay science

DIY Mouse IFN Lambda 2/3 (IL-28A/B) ELISA

Catalog No. 62830

Assay Range: 31.25 - 2000 pg/ml

Store **all** components at 2 - 8°C

The standard in this kit is Lambda 3 (IL-28B).
This kit detects both Lambda 2 and 3 with similar reactivity.

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INTRODUCTION

IL-28A, IL-28B and IL-29 are a recently discovered class II cytokine family that displays similar properties to type I interferons (IFNs).[1,2] Similarly, they have been shown to function by signaling through the JAK-STAT pathway, up-regulating the expression of genes involved in controlling viral replication and cellular proliferation. Consequently, they have also been described in the literature as lambda IFNs (IFN-λ1[IL-29], IFN-λ2 [IL-28A] and IFN-λ3 [IL-28B]) or collectively as type III IFNs. Additional studies have shown that both type I and type III IFNs are upregulated during viral infection, suggesting each may have similar and perhaps distinct roles in controlling the host response to pathogens.[3]

All type III IFNs signal through a heterodimer receptor complex comprised of the IL-10R2 and the IL-28αR receptor chains to initiate the signal transduction cascade. In contrast, all the type I IFNs (α, β, ε, κ, ω) initiate signaling by binding to the IFNAR1/ IFNAR2 receptor complex to promote signaling. IFNAR gene knockout studies have indicated that type III IFNs cannot effectively maintain a potent antiviral response. In contrast, IL-28αR knockouts showed little effect on the overall antiviral response, suggesting that the role of type III interferons may be more selective to specific cells and viruses. However, either receptor knockout showed a strong reduction in reducing viral load when mice were treated with TLR3 and TLR9 agonists.[4] Therefore, it may be possible that the type III IFNs have been evolutionarily conserved to combat pathogens that target specifically the IFNAR receptor complex or other unique aspects of the classical type I IFN pathways.[5]

MATERIALS PROVIDED

Bring all reagents to room temperature (RT), 22-25°C, before use.

Capture Antibody (Part VDY094, 1 vial) - Refer to the lot specific certificate of analysis for the amount supplied. After reconstitution, store at 2-8ºC for up to 60 days or aliquot and store at -20ºC to -80ºC for up to 6 months. Dilute in PBS without carrier protein to the working concentration indicated on the certificate.

Detection Antibody (Part VDY095, 1 vial) - Refer to the lot specific certificate of analysis for the amount supplied. Reconstitute each vial with 1 ml of Reagent Diluent. After reconstitution, store at 2-8ºC for up to 60 days or aliquot and store at -20ºC to -80ºC for up to 6 months. Dilute in Reagent Diluent to the working concentration indicated on the certificate .

Standard (Part VDY096, 3 vials) - Refer to the lot specific certificate of analysis for the amount supplied. Reconstitute each vial with 0.5 ml of Reagent Diluent. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Store reconstituted standard at 2-8ºC for up to 60 days or aliquot and at -70ºC for up to 2 months. A seven point standard curve using 2-fold serial dilutions in Reagent Diluent, and a high standard of 2000 pg/ml is recommended.

Streptavidin-HRP (Part VDY803, 1 vial) - 1.0 ml of streptavidin conjugated to horseradish-peroxidase. Store at 2-8ºC for up to 6 months after initial use. Do not freeze. Dilute to the working concentration specified on the vial label using Reagent Diluent (See Solutions Required section).

SOLUTIONS REQUIRED (NOT PROVIDED)

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 - 7.4, 0.2 µm filtered.

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2 - 7.4

Reagent Diluent - 1% BSA in PBS, pH 7.2 - 7.4, 0.2 µm filtered (Quality of BSA is critical, see Technical Hints and Limitations on pg. 9)

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine, TMB)

Stop Solution - 2 N H_2SO_4

Specifications: This DIY ELISA is a Do-It-Yourself Development kit that contains the basic components necessary for the development of sandwich ELISAs to measure natural and recombinant Mouse Interferon Lambda 2/3 (IL-28A/28B). DIY kits are intended for the analysis of cell culture supernates. Other matrices need to be evaluated on a case-by-case basis. When used according to instructions and recommended materials, each DIY kit contains sufficient materials to develop approximately fifteen 96-well ELISA plates.

Specificity: This kit exhibits 100% cross-reactivity to recombinant Mouse IFN-λ2 (IL-28A) and to recombinant Mouse IFN-λ3 (IL-28B).

Storage: For retention of full activity, all reagents should be kept at 2-8°C in the dark. Deionized or distilled water should be used for preparation of all reagents. All dilutions should be made with polypropylene tubes and pipette tips. Pipette tips should be changed between each dilution tube.

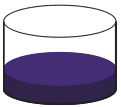
Precaution: The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.

For laboratory research use only. Not for use in diagnostic or therapeutic procedures.

ASSAY PROCEDURE - QUICK REFERENCE

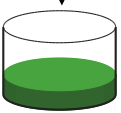
(After plate preparation procedures, pg. 7)

Total Time: 4 hr, 40 min



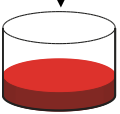
Add **100 µl** Standard, Sample or Blank

Incubate **2 hrs**
Aspirate and wash **3x**



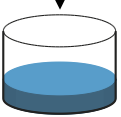
Add **100 µl** Diluted Detection Ab

Incubate **2 hrs**
Aspirate and wash **3x**



Add **100 µl** Diluted HRP Solution

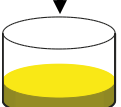
Incubate **20 min** in the dark
Aspirate and wash **3x**



Add **100 µl** Substrate Solution

Incubate **20 min** in the dark
Do not seal or wash.

Note: ALL incubations are at room temperature (22 - 25ºC)



Add **50 µl** Stop Solution
Read plate within 5 min
(450 nm)

GENERAL ELISA PROTOCOL

PLATE PREPARATION

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µl per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature (RT), 22-25ºC.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (400 µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate.
3. Block plates by adding 300 µl of Reagent Diluent to each well. Incubate at RT (22-25ºC) for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

ASSAY PROCEDURE

1. Add 100 µl of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with a plate sealer and incubate 2 hours at RT (22-25ºC).
2. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
3. Add 100 µl of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new plate sealer and incubate 2 hours at RT (22-25ºC.
4. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
5. Add 100 µl of the working dilution of Streptavidin-HRP to each well. Cover the plate with a new plate sealer and incubate for 20 minutes at RT (22-25ºC). Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 µl of Substrate Solution to each well. Incubate for 20 minutes at RT (22-25ºC). Avoid placing the plate in direct light.
8. Add 50 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is unavailable, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

TECHNICAL HINTS AND LIMITATIONS

- This DIY kit should not be used beyond the expiration date on the label.
- The use of high quality Bovine Serum Albumin (BSA) for the Reagent Diluent is crucial for the optimum performance of the DIY kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can inhibit or interfere with the detection of certain analytes. If the standard curve appears suppressed, consider evaluating a different preparation of BSA.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell culture supernate samples. Validate diluents for specific sample types prior to use.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate.
- Use a fresh reagent reservoir and pipette tips for each step.

- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- The use of PBL tablets may interfere in this assay.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. An alternative is to construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-28A/B concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

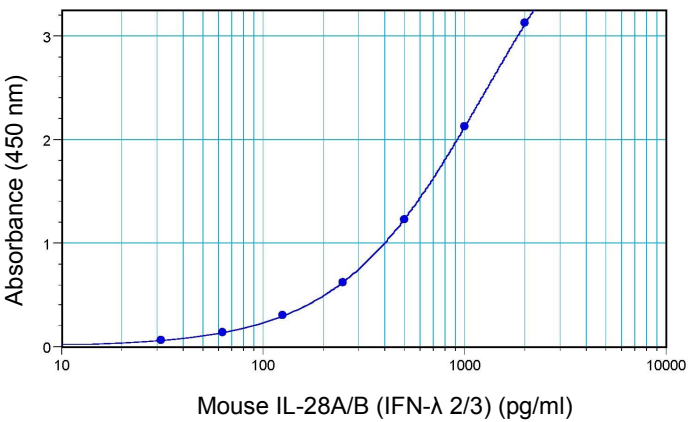
TYPICAL DATA

This standard curve is only for demonstration purposes.

A standard curve should be generated for each set of samples assayed.

The graph below represents typical data generated when using this DIY Mouse IFN Lambda 2/3 (IL-28A/B) ELISA kit. The standard curve was calculated using a computer generated 4-PL curve-fit.

Figure 1: Typical Standard Curve



CALIBRATION

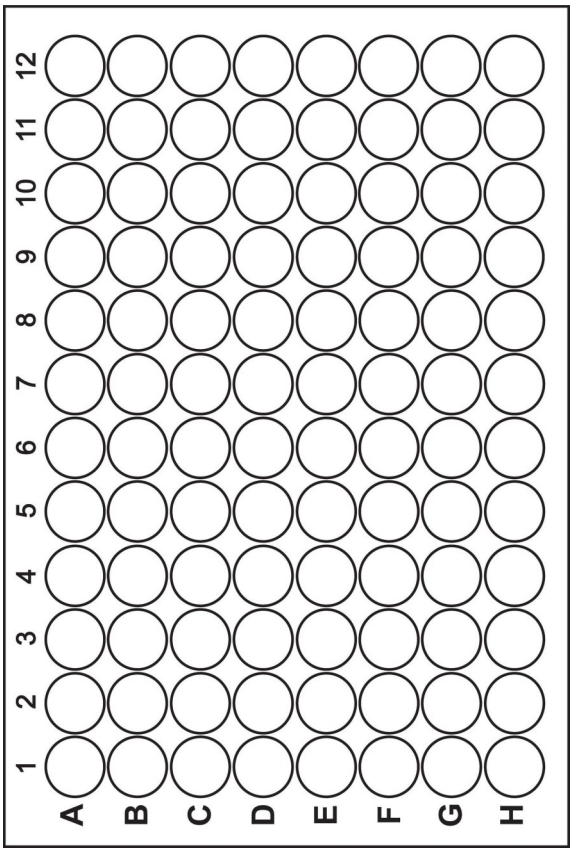
This DIY kit is calibrated against a highly purified *E.coli*-expressed recombinant mouse IL-28B/IFN-λ3 (PBL Catalog No. 12820-1).

REFERENCES

1. Sheppard, P., *et al.* (2003), *Nat. Immunol.* 4:63-68.
2. Kotenko, SV., *et al.* (2007), *Nat. Immunol* 4:69-77.
3. Onoguchi, K., *et al.* (2007), *J. Immunol.* 282:7576-7581.
4. Ank, N., *et al.* (2008), *J. Immunol.* 180:2474-2485.
5. Ank, N., *et al.* (2006), *JICR.* 26:373-379.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



NOTES

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