

## TECHNICAL HINTS AND LIMITATIONS

- This *VeriKine-DIY™* kit should not be used beyond the expiration date on the label.
- 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 samples 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 understanding of assay 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. Removing any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- 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.

## 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.

## CALCULATION OF RESULTS

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

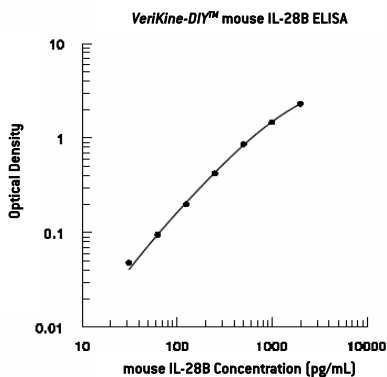
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic [4-PL] curve-fit. An alternative is to construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-28B concentrations versus the log of the O.D. 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 mouse IL-28B *VeriKine-DIY™* kit. The standard curve was calculated using a computer generated 4-PL curve-fit.



## 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

## SPECIFICITY

This kit exhibited no cross-reactivity or interference when tested with 50 ng/ml of recombinant human IL-29 or 50 ng/ml of human IL-29.

## CALIBRATION

This *VeriKine-DIY™* kit is calibrated against a highly purified E. coli expressed recombinant mouse IL-28B (PBL Catalog # 12820-1)

## FOR RESEARCH USE ONLY.

## NOT FOR USE IN THERAPEUTIC OR DIAGNOSTIC PROCEDURES.

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*VeriKine-DIY™*

Mouse

IL-28B/IFN- $\lambda$ 3 ELISA

Catalog Number: 62820-1

This *VeriKine-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 Interleukin 28B (IL-28B). *VeriKine-DIY™* kits are designed for the analysis of cell culture supernates only. When used according to instructions and recommended materials, each *VeriKine-DIY™* kit contains sufficient materials to develop approximately fifteen 96-well ELISA plates

## 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 upregulating 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- $\lambda$ 1 [IL-29], IFN- $\lambda$ 2 [IL-28A] and IFN- $\lambda$ 3 [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-28 $\alpha$ R receptor chains to initiate the signal transduction cascade. In contrast, all the type I IFNs ( $\alpha$ ,  $\beta$ ,  $\epsilon$ ,  $\kappa$ ,  $\omega$ ) initiate signaling by binding to the IFNAR1/IFNAR2 receptor complex to promote signaling. IFNAR gene knock-out studies have indicated type III IFNs cannot effectively maintain a potent antiviral response. In contrast, IL-28 $\alpha$ R 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 knock-out 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 before use.**

**Capture Antibody (Part VDY807, 1 vial)**—180  $\mu$ g/ml of rat anti-mouse IL-28B when reconstituted with 1.0 ml of PBS. After reconstitution, store at 2-8 °C for up to 60 days or aliquot and store at -20 °C to -80 °C for up to 6 months. Dilute to a working concentration of 1.0  $\mu$ g/ml in PBS, without carrier protein.

**Detection Antibody (Part VDY808, 1 vial)**—45  $\mu$ g/ml of biotinylated rat anti-mouse IL-28B when reconstituted with 1.0 ml of Reagent Diluent (See Solutions Required section). After reconstitution, store at 2-8 °C for up to 60 days or aliquot and store at -20 °C to -80 °C for up to 6 months. Dilute to a working concentration of 250 ng/ml in Reagent Diluent.

**Standard (Part VDY809, 3 vials)**—Each vial contains 120 ng/ml of recombinant mouse IL-28B when reconstituted with 0.5 ml of Reagent Diluent (See Solutions Required section). Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Store reconstituted standard at 2-8 °C for up to 60 days or aliquot and at -80 °C 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 2-8 °C 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

**PBS**—137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.2-7.4, 0.2  $\mu$ m filtered.

**Wash Buffer** — 0.05% Tween<sup>®</sup> 20 in PBS, pH 7.2-7.4 (Concentrated solution available, PBL Catalog # 60126-1)

**Reagent Diluent** — 1% BSA in PBS, pH 7.2-7.4, 0.2  $\mu$ m filtered. (Concentrated solution available, PBL Catalog # 60995-1)

**Substrate Solution** — 1:1 mixture of Color Reagent A (H<sub>2</sub>O<sub>2</sub>) and Color Reagent B (Tetramethylbenzidine, TMB) (PBL Catalog # 60999-1)

**Stop Solution** — 2 N H<sub>2</sub>SO<sub>4</sub> (PBL Catalog # 60994-1)

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## 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  $\mu$ l per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
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  $\mu$ l) 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 blotting it against clean paper towels.
3. Block plates by adding 300  $\mu$ l of Reagent Diluent to each well. Incubate at room temperature 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  $\mu$ l of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100  $\mu$ l of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100  $\mu$ l of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100  $\mu$ l of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50  $\mu$ l 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 not available, 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.