

# Equine IL-1 $\beta$ VetSet™ ELISA Development Kit



**Catalog Number:** VS0131E-002  
**Lot Number:** FK0351FK  
**Quantity:** 2 Plate Set  
**Storage:** 2-8°C  
**Sample Type:** Cell Culture Supernatants  
**Stability:** 12 months (from date of receipt)  
**Alias:** IL1F2  
**Country of Origin:** USA

Included Components:	Description	Quantity	Component Number	Lot Number
	Equine IL-1 $\beta$ Coated Plate	2 each	VS0131E-CP	FK0350LJ
	Equine IL-1 $\beta$ Standard	2 each	VS0131E-ST	FK0348CJ
	Equine IL-1 $\beta$ Detection Antibody	2 each	VS0131E-DA	FK0349BK
	Streptavidin-HRP	1 each	AR0068-001	FK0331CK
	Plate Sealer	6 each	N/A	N/A

Additional Reagents Required:	Reagent	Formulation
	DPBS	0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4
	Standard and Sample Diluent	Complete cell culture medium used to generate cell culture supernatant samples. It is critical that this medium contain at least 1% carrier protein. If the medium does not contain carrier protein, use Reagent Diluent to dilute the Standard and samples.
	Reagent Diluent	4% BSA in DPBS, 0.2 $\mu$ m filtered
	Wash Buffer	0.05% Tween®-20 in DPBS
	Substrate	3,3',5,5'-tetramethylbenzidine (TMB) Substrate <i>ELISA Accessory Pack: Catalog # AR0133-002</i>
	Stop Solution	0.18 M Sulfuric Acid <i>ELISA Accessory Pack: Catalog # AR0133-002</i>

Component Preparation:	Component	Preparation
	Equine IL-1 $\beta$ Standard	Reconstitute Standard in 500 $\mu$ L Standard and Sample Diluent. The Standard now has a concentration of <b>150 ng/mL</b> . Prepare 1:1 serial dilutions of the Standard by mixing 250 $\mu$ L Standard with 250 $\mu$ L Standard and Sample Diluent. Repeat 1:1 serial dilutions until reach a final concentration of 2.34 ng/mL. Use Standard and Sample Diluent as a zero standard.
	Equine IL-1 $\beta$ Detection Antibody Working Solution	Reconstitute Detection Antibody in 500 $\mu$ L Reagent Diluent. Dilute the 500 $\mu$ L of reconstituted Detection Antibody in 11.5 mL Reagent Diluent.
	Streptavidin-HRP Working Solution	Dilute 500 $\mu$ L of Streptavidin-HRP in 11.5 mL Reagent Diluent.

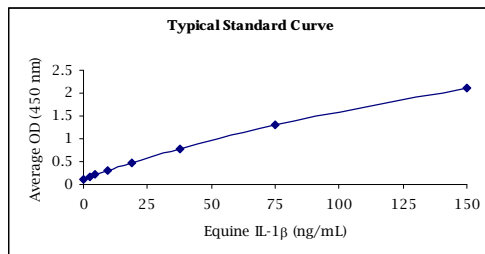
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- ELISA Procedure:**
1. Prepare Standard and cell culture supernatant sample dilutions in Standard and Sample Diluent.
  2. Add 100  $\mu$ L of Standard or sample to appropriate wells.  
Note: Run each Standard or sample in duplicate.
  3. Cover plate with Plate Sealer and incubate at room temperature (20-25°C) for 2 hours.
  4. Wash plate FOUR times with Wash Buffer.  
Note: Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.
  5. Add 100  $\mu$ L of Detection Antibody Working Solution to each well.
  6. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
  7. Wash plate FOUR times with Wash Buffer as described in step 4.
  8. Add 100  $\mu$ L of Streptavidin-HRP Working Solution to each well.
  9. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes.
  10. Wash plate FOUR times with Wash Buffer as described in step 4.
  11. Add 100  $\mu$ L of TMB Substrate Solution to each well.
  12. Develop the plate in the dark at room temperature for 30 minutes.  
Note: Do **NOT** cover plate with Plate Sealer.
  13. Stop reaction by adding 100  $\mu$ L of Stop Solution to each well.
  14. Measure absorbance on a plate reader at 450 nm.

**Typical Standard Curve:**



Data represents a typical standard curve generated using the Equine IL-1 $\beta$  VetSet™ ELISA Development Kit.

A standard curve should be generated with each assay.

**Representative Data:**

Stimulant	Equine IL-1 $\beta$ (ng/mL)
Unstimulated	< 3
Staphylococcal enterotoxin B (SEB; 5 $\mu$ g/mL)	3.6
Phytohemagglutinin (PHA; 10 $\mu$ g/mL)	18.7
Phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) and Ionomycin (500 ng/mL)	57

PBMCs harvested by ficoll density gradient from an apparently healthy equine were suspended in RPMI medium containing 10% fetal bovine serum and stimulated as desired. The cell-free supernatants were harvested following three days stimulation and analyzed in the Equine IL-1 $\beta$  VetSet™ ELISA Development Kit.

**Technical Notes:** This kit is for the quantitative measurement of Equine IL-1 $\beta$  in cell culture supernatants. If assaying other sample types, an appropriate Sample and Standard Diluent will need to be developed and validated. Any changes to the ELISA protocol may significantly affect the results generated and will require optimization.

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