Canine IL-1β ELISA Reagent Set

NB-06-1137

FOR RESEARCH USE ONLY

Technical Notes:	This kit is for the quantitative measurement of Canine IL-1β 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.	
Included Components:	**Description**	**Quantity**
	Canine IL-1β Coated Plate	2 each
	Canine IL-1β Standard	2 each
	Canine IL-1β Detection Antibody	2 each
	Streptavidin-HRP	1 each
	Plate Sealer	6 each
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 µm filtered
	Wash Buffer	0.05% Tween®-20 in DPBS
	Substrate	3,3',5,5'-tetramethylbenzidine (TMB) Substrate
	Stop Solution	0.18 M Sulfuric Acid
Component Preparation:	**Component**	**Preparation**
	Canine IL-1β Standard	Reconstitute Standard in 1 mL Standard and Sample Diluent. Dilute 139 µl of the reconstituted standard in 861 µl of Standard and Sample Diluent. The Standard now has a concentration of **2.5 ng/ml** Prepare 1:1 serial dilutions of the Standard by mixing 250 µL Standard with 250 µL Standard and Sample Diluent. Repeat 1:1 serial dilutions until reach a final concentration of **0.039** ng/mL. Use Standard and Sample Diluent as a zero standard.
	Canine IL-1β Detection Antibody Working Solution	Reconstitute Detection Antibody in 500 µL Reagent Diluent. Dilute the 500 µL of reconstituted Detection Antibody in 11.5 mL Reagent Diluent.
	Streptavidin-HRP Working Solution	Dilute 500 µL of Streptavidin-HRP in 11.5 mL Reagent Diluent.

ELISA Procedure:

1. Prepare Standard and cell culture supernatant sample dilutions in Standard and Sample Diluent.
2. Add 100 µL 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 1 hour.
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 µL 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 µL of Streptavidin-HPR 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 µL 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 µL of Stop Solution to each well.
14. Measure absorbance on a plate reader at 450 nm.

Representative Data:

Stimulant	Canine IL-1β (ng/ml)
Unstimulated	1.3
Staphylococcal enterotoxin B (SEB; 5 µg/mL)	11.6
Phytohemagglutinin (PHA; 10 µg/ml)	6.6
Phobol 12-myristate 13-acetate (PMA; 10 ng/ml) and Ionomycin (500 ng/ml)	7.4

PBMCs harvested by ficoll density gradient from an apparently healthy canine were suspended in RPMI medium containing 10% fetal bovine serum and stimulated as desired. The cell-free supernatants were harvested following six days stimulation and analyzed in the Canine IL-1β ELISA Development Kit.

Country of Origin: USA