

Swine CCL2 VetSet™ ELISA Development Kit



Catalog Number: VS0081S-002
Lot Number: KK0475HJ
Quantity: 2 Plate Set
Storage: 2-8°C
Sample Type: Cell Culture Supernatants
Stability: 12 months (from date of receipt)
Alias: MCP-1
Country of Origin: USA

Included Components:	Description	Quantity	Component Number	Lot Number
	Swine CCL2 Coated Plate	2 each	VS0081S-CP	KK0441DJ
	Swine CCL2 Standard	2 each	VS0081S-ST	GJ0139II
	Swine CCL2 Detection Antibody	2 each	VS0081S-DA	HJ0163DJ
	Streptavidin-HRP	1 each	AR0068-001	HJ0166HI
	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 µm 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
	Swine CCL2 Standard	Reconstitute Standard in 1 mL Standard and Sample Diluent. The Standard now has a concentration of 1200 pg/mL . Prepare 1:1 serial dilutions of reconstituted Standard by mixing 250 µL reconstituted Standard with 250 µL Standard and Sample Diluent. Repeat 1:1 serial dilutions until reach a final concentration of 18.75 pg/mL. Use Standard and Sample Diluent as a zero standard.
	Swine CCL2 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.

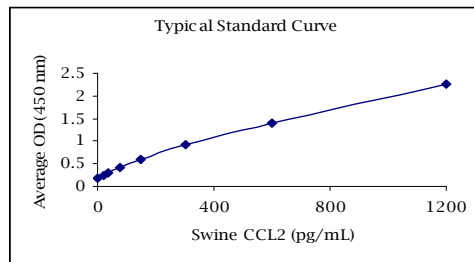
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- ELISA Procedure:**
1. Prepare Standard and cell culture supernatant sample dilutions in Standard and Sample Diluent.
 2. Add 100 μ L of Standard or sample to appropriate wells.
Note: Run each Standard or sample in duplicate.
 3. Cover plate with Plate Sealer and incubate at room temperature (20-25°C) for 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 μ 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 μ 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 μ 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 μ 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 Swine CCL2 VetSet™ ELISA Development Kit.

A standard curve should be generated with each assay.

Representative Data:

Stimulant	Swine CCL2 (pg/mL)
Unstimulated	1,918
Staphylococcal enterotoxin B (SEB; 5 μ g/mL)	121,703
Phytohemagglutinin (PHA; 10 μ g/mL)	77,950
Phorbol 12-myristate 13-acetate (PMA; 10 ng/mL) and Ionomycin (500 ng/mL)	692

PBMCs harvested by ficoll density gradient from an apparently healthy porcine were suspended in RPMI medium containing 10% serum and stimulated as desired. The cell-free supernatants were harvested following three days stimulation and analyzed in the Swine CCL2 VetSet™ ELISA Development Kit.

Technical Notes: This kit is for the quantitative measurement of Swine CCL2 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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