



## Canine TNF- $\alpha$ /TNFSF1A ELISpot Development Module

Catalog Number: SEL1507

### Reagents Provided

**Canine TNF- $\alpha$  Capture Antibody Concentrate** (Part # 843585)-  
1 vial of lyophilized anti-canine TNF- $\alpha$ .\*

**Canine TNF- $\alpha$  Detection Antibody Concentrate** (Part # 843586)-  
1 vial of lyophilized biotinylated anti-canine TNF- $\alpha$ .\*

\*Each vial contains sufficient antibodies to run ELISpot assays on approximately five 96-well microplates, when using the protocol provided.

### Other Supplies Required

- ELISpot Blue Color Module or equivalent (R&D Systems, Catalog # SEL002)
- 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 - (*i.e.* 0.05% Tween<sup>®</sup> 20 in PBS).
- Blocking Buffer - (*i.e.* 1% BSA, 5% Sucrose in PBS).
- Reagent Diluent - (*i.e.* 1% BSA in PBS, pH 7.2 - 7.4, 0.2  $\mu$ m filtered).
- 37° C CO<sub>2</sub> incubator.
- Deionized H<sub>2</sub>O.
- Positive Control - Use recombinant canine TNF- $\alpha$  (R&D Systems, Catalog # 1507-CT) or cells known to secrete canine TNF- $\alpha$ .
- 96-well plates - Nitrocellulose-bottom plates, PVDF-bottom Immunospot<sup>®</sup> plates, or flat-bottom polystyrene Immulon<sup>®</sup> ELISA plates.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Dissection microscope or an automated ELISpot Reader.

### ELISpot Protocol

When a 96-well PVDF microplate is used, a 1:60 dilution of the Capture and Detection antibodies is recommended. **Each investigator should determine the optimal working dilution of the antibodies depending on the type of microplate, Wash Buffer and Blocking Buffer used.**

1. Calculate the total volume of Capture Antibody needed and dilute to the working concentration using PBS.
2. Immediately add 100  $\mu$ L of the diluted Capture Antibody per well. Cover the plate with a lid and incubate overnight at 2 - 8° C.
3. Aspirate Capture Antibody from each well and wash 3 times with Wash Buffer or PBS (350  $\mu$ L/well) using either a squirt bottle, manifold dispenser, or autowasher. After the final wash, remove any remaining liquid by inverting the plate and blotting it against a clean paper towel.  
*Do not touch the membranes during washing to avoid damage.*
4. Block membranes by adding 200  $\mu$ L of Blocking Buffer to each well. Incubate for 2 hours at room temperature.
5. Aspirate Blocking Buffer as described in step 3. Rinse with the same media in which the cells will be cultured.  
*Do not discard the culture media until cells are ready to be plated.*
6. Aspirate culture media from the plate and immediately fill appropriate wells with 100  $\mu$ L of culture media containing canine TNF- $\alpha$  secreting cells. Incubate at 37° C in a 5% CO<sub>2</sub> incubator. Incubation time must be determined empirically.  
*We recommend running a positive control (recombinant protein), negative control (same number of unstimulated cells as stimulated cells), and background control (sterile culture media) with each assay.*
7. Wash plate 4 times with Wash Buffer. Remove any remaining Wash Buffer by inverting the plate and blotting it against a clean paper towel.
8. Calculate the total volume of Detection Antibody needed and dilute to the working concentration using Reagent Diluent.
9. Add 100  $\mu$ L of the diluted Detection Antibody per well. Cover the plate with a lid and incubate overnight at 2 - 8° C.
10. Aspirate Detection Antibody and wash as described in step 3. Microplates are ready for color development.

### Color Development

Color development may be done using the ELISpot Blue Color Module that may be purchased separately. Alternatively, another chromogen of choice may be used.

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**R&D Systems, Inc.**

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