



## ***Rat TNF- $\alpha$ ELISpot Development Module***

Catalog Number: SEL510

### ***Reagents Provided***

**Rat TNF- $\alpha$  Capture Antibody Concentrate** (Part # 841542) - 1 vial of lyophilized hamster anti-mouse TNF- $\alpha$  monoclonal antibody.\*

**Rat TNF- $\alpha$  Detection Antibody Concentrate** (Part # 841543) - 1 vial of lyophilized biotinylated goat anti-mouse TNF- $\alpha$  polyclonal antibody.\*

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

### ***Reagent Preparation and Storage***

**Capture Antibody Concentrate** - Reconstitute with 1 mL of PBS. After reconstitution, store at 2 - 8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.

**Detection Antibody Concentrate** - Reconstitute with 1 mL of Reagent Diluent. After reconstitution, store at 2 - 8° C for up to 60 days or aliquot and store at -20° C to -70° C in a manual defrost freezer for up to 6 months.

**For optimal performance, prepare the working dilutions of the Capture and Detection Antibodies immediately before use.**

### ***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 - 0.05% Tween<sup>®</sup> 20 in PBS.
- Blocking Buffer - 1% BSA, 5% Sucrose in PBS.
- Reagent Diluent - 1% BSA in PBS, pH 7.2 - 7.4, 0.2  $\mu$ m filtered.
- 2 - 8° C refrigerator.
- 37° C CO<sub>2</sub> incubator.
- Positive Control - Use recombinant rat TNF- $\alpha$  (R&D Systems, Catalog # 510-RT) or cells known to secrete rat TNF- $\alpha$ .
- 96-well plates - Nitrocellulose-bottom plates, PVDF-bottom Immunospot<sup>®</sup> plates, or flat-bottom polystyrene Immulon<sup>®</sup> ELISA plates.
- Squeeze bottle, manifold dispenser, or automated microplate washer.
- Dissection microscope or an automated ELISpot Reader.
- Deionized H<sub>2</sub>O.

### ***ELISpot Protocol***

When a 96-well PVDF microplate is used, 1:60 dilutions of the Capture and Detection Antibodies are 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 the 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 squeeze 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. 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 rat 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 the 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 the 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.

*All trademarks and registered trademarks are the property of their respective owners.*

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**R&D Systems, Inc.**  
**1-800-343-7475**