



Equine TNF- α /TNFSF1A ELISpot Development Module

Catalog Number: SEL1814

Reagents Provided

Equine TNF- α Capture Antibody Concentrate (Part # 842981) - 1 vial of lyophilized mouse anti-equine TNF- α monoclonal antibody.*

Equine TNF- α Detection Antibody Concentrate (Part # 842982) - 1 vial of lyophilized biotinylated goat anti-equine TNF- α polyclonal antibody.*

*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 Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2 - 7.4, 0.2 μ m filtered.
- Wash Buffer - 0.05% Tween[®] 20 in PBS.
- Blocking Buffer - 1% BSA, 5% Sucrose in PBS.
- Reagent Diluent - 1% BSA in PBS, pH 7.2 - 7.4, 0.2 μ m filtered.
- 2 - 8° C refrigerator.
- 37° C CO₂ incubator.
- Deionized H₂O.
- Positive Control - Use recombinant equine TNF- α (R&D Systems, Catalog # 1814-ET) or cells known to secrete equine TNF- α .
- 96-well plates - Nitrocellulose-bottom plates, PVDF-bottom Immunospot[®] plates, or flat-bottom polystyrene Immulon[®] 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, 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 μ 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 μ 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 μ L of Blocking Buffer to each well. Cover the plate with the lid and 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 μ L of culture media containing equine TNF- α secreting cells. Cover the plate with the lid and incubate at 37° C in a 5% CO₂ 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 μ 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.

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