Equine IL-2 ELISpot Development Module

Catalog Number: SEL1613

Reagents Provided

Equine IL-2 Capture Antibody Concentrate (Part # 843045) - 1 vial of lyophilized goat anti-equine IL-2 polyclonal antibody.*

Equine IL-2 Detection Antibody Concentrate (Part #843046) - 1 vial of lyophilized biotinylated goat anti-equine IL-2 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 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 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_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 - 7.4, 0.2 μm 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 μm filtered.
- 2 - 8° C refrigerator.
- 37° C CO_2 incubator.
- Deionized H_2O.
- Positive Control - Use recombinant equine IL-2 (R&D Systems, Catalog # 1613-IL) or cells known to secrete equine IL-2.
- 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, 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 μL 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 μL/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 μL 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 μL of culture media containing equine IL-2 secreting cells. Incubate at 37° C in a 5% CO_2 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 μL 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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