Canine IL-2 Development Module

Catalog Number: SEL1815

Reagents Provided

Canine IL-2 Capture Antibody Concentrate (Part # 843475) - 1 vial of lyophilized anti-canine IL-2.*

Canine IL-2 Detection Antibody Concentrate (Part # 843476) - 1 vial of lyophilized biotinylated anti-canine IL-2.*

*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 - (*i.e.* 0.05% Tween® 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 μm filtered).
- 37° C CO_2 incubator.
- Deionized H_2O.
- Positive Control - Use recombinant canine IL-2 (R&D Systems, Catalog # 1815-CL) or cells known to secrete canine IL-2.
- 96-well plates - Nitrocellulose-bottom plates, PVDF-bottom Immunospot® plates, or flat-bottom polystyrene Immulon® ELISA plates.
- Multi-channel pipette, 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, multi-channel pipette, 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 canine 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. See next page for Color Development protocol.

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ELISpot Blue Color Module

Catalog Number: SEL002

Reagents Provided and Storage

Streptavidin-AP Concentrate (Part # 845100) - 1.0 mL of Streptavidin conjugated to Alkaline Phosphatase. Store at 2 - 8° C. **DO NOT FREEZE.**

BCIP/NBT Chromogen (Part # 895866) - 50 mL of 5-Bromo-4-Chloro-3′ Indolylphosphate p-Toluidine Salt (BCIP) and Nitro Blue Tetrazolium Chloride (NBT) in organic solvent. Store at 2 - 8° C

Precautions

Although the toxicity of the chromogenic substrate BCIP/NBT is not currently known, wear gloves to avoid contact with skin. Follow local, state and federal regulations to dispose of used BCIP/NBT.

Color Development Protocol

1. Calculate the total volume of Streptavidin-AP needed and dilute Streptavidin-AP Concentrate with Reagent Diluent to a working dilution of 1:60.
2. Add 100 µL of the diluted Streptavidin-AP into each well and incubate for 2 hours at room temperature.
3. Wash the plate 3 times with Wash Buffer. Rinse again with deionized water, then remove excess water by inverting the plate and blotting it against a clean paper towel.
4. Add 100 µL of BCIP/NBT solution into each well. Cover the plate and incubate in the dark for 30 minutes at room temperature.
5. Rinse with deionized water. Invert plate and tap to remove excess water and allow the plate to dry at room temperature or at 37° C.
6. Spots can be quantified manually using a dissection microscope or automatically by using a specialized automated ELISpot reader.

Limitations of ELISpot Reagents

- A basic understanding of ELISpot assay development is required for the successful use of these reagents. The protocol provided is for demonstration purposes only. The type of enzyme and substrate and the concentrations of capture/detection antibodies used can give varied results.
- Individual results may vary due to differences in technique, plasticware and water sources.
- Working dilutions should be prepared and used immediately.
- Each investigator should optimize the experimental conditions, such as cell type, cell stimulation conditions and cell dilutions of the assay.
- Reagents should not be used beyond the expiration date on the label.