

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) - 2 vials (25 mL/vial) 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.

Other Supplies Required

- PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2 - 7.4, 0.2 µm filtered.
- Reagent Diluent - (*i.e.* 1% BSA in PBS, pH 7.2 - 7.4, 0.2 µm filtered).
- Wash Buffer - (*i.e.* 0.05% Tween[®] 20 in PBS).
- Deionized water.

Precautions

The BCIP/NBT Chromogen is highly flammable and toxic if swallowed, in contact with skin, or inhaled. Before use, refer to the MSDS, which can be found at www.RnDSYSTEMS.com.

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, and 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.

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