Andy Fluor™ 488 Streptavidin

SKU#: VB-L156B

Description

Andy Fluor™ 488 streptavidin is prepared by covalently labeling streptavidin with Andy Fluor™ 488 dye. Streptavidin has high binding affinity with biotin, so streptavidin conjugate is commonly used together with biotin conjugate for specific detection of a variety of proteins, protein motifs, nucleic acids, and other molecules. Strategies similar to this are used in many detection protocols including western blots, flow cytometry, imaging and microscopy, and microplate assays.

Specification

Physical State	Lyophilized powder
Buffer	PBS, pH 7.4
Preservative	0.02% sodium azide
Stabilizer	0.1% BSA
Label	Andy Fluor™ 488
Excitation/Emission	495/520 nm

Package Size

500 µg (Lyophilized powder)

Reconstitution and Storage:

Store lyophilized powder at 2-8ºC. When ready to use, rehydrate with dH2O (50 µL for 100 µg antibody or 250 µL for 500 µg antibody) to make 2 mg/mL solution and centrifuge if not clear. Product is stable for about 6 months at 2-8ºC as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, add an equal volume of glycerol for a final concentration of 50%, and store at -20 ºC as a liquid.

Guidelines for Use

Centrifuge the reconstituted protein conjugate solution briefly in a microcentrifuge before use. Add only the supernatant to the experiment. This step eliminates any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically.

For the fluorophore-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemical applications. For flow cytometry applications, 0.06–1.0 µg per 1×10^6 cells should yield satisfactory result.

General Protocols

Immunofluorescence Protocol for Microscopy

1. **Preparation of Slides**

For Cultured Cells

- Grow cultured cells on sterile glass cover slips or slides overnight at 37 º C
- Wash briefly with PBS
- Fix as desired. Possible procedures include: a) 20 minutes with 10% formalin in PBS (keep wet); 2) 10 minutes with ice cold methanol, allow to air dry; 3) 10 minutes with ice cold acetone, allow to air dry
- Wash in PBS

For Frozen Sections

- Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store the frozen tissue block at -80°C until ready for sectioning.
- Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
- Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.
- Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).
- Sections can be stored in a sealed slide box at -80°C for later use.
- Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone or ice cold methanol for 10 minutes. Air dry for 30 minutes.
- Wash in PBS

For Paraffin Sections

- Deparaffinize sections in xylene, 3×5min.
- Hydrate with 00% ethanol, 2×2min.
- Hydrate with 95% ethanol, 2×2min.
- Rinse in distilled water.
- Follow procedure for pretreatment as required.

2. **Antigen retrieval**

Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining can proceed. Heat-mediated and enzymatic antigen retrievals are common methods.

- **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer, pH 6.0; maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
- **For EDTA:** Bring slides to a boil in 1 mM EDTA, pH 8.0: follow with 15 minutes at a sub-boiling temperature. No cooling is necessary.

- **For TE:** Bring slides to a boil in 10 mM TE/1 mM EDTA, pH 9.0: then maintain at a sub-boiling temperature for 18 minutes. Cool at room temperature for 30 minutes.
- **For Pepsin:** Digest for 10 minutes at 37°C.

Note: Generally it is not necessary to pretreat frozen sections or cultured cells with above antigen retrieval protocol.

3. **Staining Procedure**

- Rinse sections in PBS-Triton X-100 (0.025%) for 2×2min
- **Serum Blocking**: incubate sections with 3-4 drops of RTU normal horse serum for 30 minutes to block non-specific binding of immunoglobulin.
- **Primary Antibody**: incubate sections with primary antibody (rabbit or mouse IgG) at appropriate dilution in antibody dilution buffer (SKU#: VB-6002) for 1-2 hour at room temperature or overnight at 4 °C. Rinse in PBS.
- **Secondary Antibody**: incubate sections with 3-4 drops of RTU biotinylated anti-rabbit or mouse secondary antibody for 30 minutes at room temperature.
- Rinse in PBS for 3x2min.
- **Detection**: incubate sections with 3-4 drops of RTU **Andy Fluor™ 488 streptavidin** for 30 minutes at room temperature.
- Rinse in PBS for 3×2min.
- Count stain with Dapi (blue fluorescence) or other fluorescence nuclei stain dye (red color).
- Mount with aqueous mounting medium
- The slides can be **visualized** under fluorescence microscope using the correct filter.
- Store slides in the dark at 4°C.