Spot-Label for Immunofluorescence of Spot-Tag® Fusion Proteins

Only for research applications, not for diagnostic or therapeutic use

1. Introduction

Small peptide tags are useful for the labelling and detection of proteins using immunostaining, immunoblotting or immunoprecipitation techniques. The ChromoTek Spot-Tag is a short 12 amino acid affinity tag PDRVRAVSHWSS, which can be cloned either N- or C-terminally to a protein of interest. This tag can be efficiently immunostained with the novel Spot-Label affinity reagent. The Spot-Label consists of a small recombinant bivalent alpaca single-domain antibody fragment covalently coupled to a fluorescent dye. Due to its small size, immunostaining of the Spot-Tag with the Spot-Label minimizes the "linkage error" for super-resolution microscopy applications (e.g. STED and dSTORM). In addition, the Spot-Label has a superior tissue penetration rate, better access to the Spot epitope, and higher labelling density.

2. Content

Reagent	Quantity	Code
Spot-Label ATTO594 for Immunofluorescence (IF), bivalent	50 µl	eba594-50
Spot-Label ATTO594 for Immunofluorescence (IF), bivalent	10 µl	eba594-10

Concentration: 1 g/L. Storage buffer: 1x PBS, 0.09% sodium azide.

3. Optical Properties

ATTO 594: Excitation range 580 - 615 nm (λ_{abs}= 601 nm)
Emission range 620 - 660 nm (λ_{fl}= 627 nm)

For further information please refer to http://www.atto-tec.com

4. Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C / 40°F.
Stable for 6 months. Do not freeze. Protect from light.

5. IF Protocol

1. **Fixation**: Fix cells seeded on coverslips in 3.7% formaldehyde in PBS for 10 min at room temperature.

   Note: Always prepare a fresh formaldehyde dilution.

2. Wash samples three times with PBS (Phosphate Buffered Saline). Do not store fixed cells.
3. **Permeabilization:** Add PBS containing 0.5% Triton X-100 to samples and incubate for 5 min at room temperature.

   Note: Alternatively, use ice-cold 100% methanol for permeabilization.

4. Wash samples twice with PBS.
5. **Blocking**: Add 4% BSA in PBS to samples and incubate for 20 min at room temperature.

   Note: If necessary, use additional blocking reagents (e.g. 10% normal serum in PBS or Image-iT™ FX Signal Enhancer from ThermoFischer Scientific) and extend the blocking time up to 60 min.

6. **Spot-Label incubation**: Dilute Spot-Label 1:2000 in blocking buffer and incubate for overnight at +4°C.

   Note: For multiplexing protocols, you can combine Spot-Label with another primary or secondary antibody.

7. Wash samples three times for 5-10 min in PBS.
8. If required, counterstain with DNA fluorescent dyes, e.g. DAPI in PBS. Proceed with imaging directly or mount samples, if necessary.
9. **Mounting:** Rinse sample briefly in water to prevent salt crystal formation. Mount in ProLong™ Diamond Antifade Mountant from ThermoFischer Scientific or other mounting media with anti-fading agents.

Suggested buffer composition

Buffer	Composition
Fixation buffer	3.7% formaldehyde in PBS
Permeabilization buffer	PBS; 0.5% Triton X-100
Wash buffer	PBS
Blocking buffer	4% BSA (w/v); PBS

Support/ Troubleshooting

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

Related Products

Spot-Tag Toolbox	Code
Spot-Trap® Agarose	eta-20
Spot-Trap® Magnetic Agarose	etma-20
Blocked agarose beads	bab-20
Spot-Label, uncoupled, for WB & IP, monovalent	etx-10; etx-250
Spot-Tag peptide	ep-1
Spin columns	sct-10; sct-20; sct-50

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