Spot-Label ATTO 488

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 (sequence: 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 conjugated to a fluorescent dye. It enables the fluorescence-based Western-blot detection and immunofluorescence microscopy analysis of Spot-Tag fusion proteins. Due to the small size of the Spot-Label, 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 ATTO 488	50 µL	eba488-50
Spot-Label ATTO 488	10 µL	eba488-10

3. Properties

Description:
Recombinant alpaca single-domain antibody for the analysis of Spot-Tag fusion proteins.

Specificity:
This antibody fragment is reactive against the Spot-Tag (PDRVRAVSHWSS).

Product Type:
Primary antibody, conjugated to ATTO 488

Isotype:
V_HH (Nanobody), alpaca monoclonal, bivalent

Purity:
Affinity-purified antibody fragment

Form:
Liquid

Storage Buffer:
Buffered aqueous solution (PBS)

Preservative:
0.09% Sodium Azide

Safety datasheet (SDS) for this product:
Sodium Azide SDS

Concentration:
1 g/L

Optical properties:
ATTO 488: Excitation range 480 - 510 nm (λ_{abs}= 501 nm)
Emission range 520 - 560 nm (λ_{fl}= 523 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 1:1,000 - 10,000 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

6. Western blot

1. **Preparation:** Separate your sample of interest on an SDS-PAGE gel and transfer onto a nitrocellulose membrane according to standard protocols.

2. **Blocking:** Incubate membrane with 5 % milk powder in PBS or TBS + 0.075 % Tween-20 (PBST or TBST).

3. **Spot-Label incubation:** Dilute Spot-Label in 5 % milk powder in PBST or TBST. The recommended starting dilution is 1:5,000. Add diluted Spot-Label to membrane and incubate at 4 °C overnight.

   Note: The optimal dilution depends on the application and should be determined by the user. A titration from 1:1,000 up to 1:20,000 is recommended.

4. Wash samples three times for 5-10 min in PBS or TBST.
5. **Detection:** Image fluorescence using a fluorescence scanner or similar and using appropriate settings

   Note: Preprogrammed imaging settings for fluorescein, AlexaFluor 488, or Cy2 will work also for ATTO 488.

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
Binding control agarose beads	bab-20
Binding control magnetic agarose beads	bmab-20
Spot V_HH, recombinant binding protein	etb-250
Spot-Label ATTO 594	eba594-50
Spot-Tag peptide	ep-1
Spin columns	sct-10; sct-20; sct-50

Trademarks and Copyrights