# Spot-Label ATTO 594

*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 594 | 50 µL | eba594-50 |
| Spot-Label ATTO 594 | 10 µL | eba594-10 |

## 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 594

**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 594:** Excitation range 580 - 615 nm ($\lambda_{abs}$= 601 nm)
Emission range 620 - 660 nm ($\lambda_{fl}$= 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 1:1,000 – 1:10,000 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 1:1,000. 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 1:10,000 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 AlexaFluor 594 or Cy3.5 will work also for ATTO 594.*

**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 488 | eba488-50 |
| Spot-Tag peptide | ep-1 |
| Spin columns | sct-10; sct-20; sct-50 |

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