RFP-Booster ATTO 594

For the immunofluorescence detection of RFP-fusion proteins in fixed cells.

1. Product

The RFP-Booster ATTO 594 is an anti-RFP Nanobody coupled to ATTO 594.

2. Introduction

Red fluorescent proteins (RFP) and its variants are widely used to study protein localization and dynamics in cells. However, photo-stability and quantum efficiency of RFP are often not sufficient for e.g. super-resolution microscopy (such as 3D-SIM or dSTORM) and for fixed cell samples. In addition, many cell biological methods such as BrdU-staining, EdU-Click-iT™ treatment or fluorescent *in situ* hybridization result in disruption of the RFP signal. The RFP-Booster reactivates, enhances, and stabilizes the RFP-signal.

3. Properties

Product size	rba594-10: 10 µL rba594-100: 100 µL
Format	Alpaca single domain antibody, Nanobody or V_HH; monovalent
Target/ Specificity	RFP and RFP variants. See www.ptglab.com for a list of recognized RFP variants.
Conjugate	Site-directed conjugation to ATTO 594
Excitation/ Emission	Excitation max: 580-615 nm, Emission max: 620-660 nm
DOL	2 fluorophores per Nanobody
Purity	Recombinantly expressed and purified
Form	Buffered aqueous solution
Storage buffer	10 mM HEPES pH 7.0, 500 mM NaCl, 5 mM EDTA, Preservative: 0.09% sodium azide, Safety datasheet (SDS): sodium azide
Concentration	0.5 g/L
Stability and storage	Shipped at ambient temperature. Store at -20°C/-4°F. Avoid freeze-thaw cycles. Aliquot upon arrival. Protect from light. Stable for 6 months.

4. 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.
   Note: Alternatively, use methanol for fixation: Apply ice-cold 100% methanol to cells for 3 min, wash as in step 2 and proceed directly with step 5 of this protocol.

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.
4. Wash samples twice with PBS.
5. **Blocking**: Add 4% BSA in PBS to samples and incubate for 10 min at room temperature.
6. **RFP-Booster incubation**: Dilute RFP-Booster 1:200 in blocking buffer and incubate for 1 h at room temperature. Optimal dilution is application-dependent and should be determined.

   Note: For multiplexing protocols, you can combine RFP-Booster with any other antibody.

7. Wash samples three times for 5-10 min in PBS.

8. If required, counter stain with DNA fluorescent dyes, e.g. DAPI in PBS.
9. **Mounting:** Rinse sample shortly in water to prevent formation of salt crystals. Mount in VECTASHIELD® Antifade Mounting Medium or other mounting media with anti-fading agents and seal mounted coverslips with clear nail polish.

Suggested buffer composition

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

Only for research applications, not for diagnostic or therapeutic use.