

## RFP-Booster ATTO 647N

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

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- 1. Product** The RFP-Booster ATTO 647N is an anti-RFP Nanobody coupled to ATTO 647N.
- 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**
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|-----------------------|---|
| Product size          | rba647n-10: 10 µL<br>rba647n-100: 100 µL  |
| Format                | Alpaca single domain antibody, Nanobody or V <sub>H</sub> H; monovalent   |
| Target/ Specificity   | RFP and RFP variants. See <a href="http://www.ptglab.com">www.ptglab.com</a> for a list of recognized RFP variants.                           |
| Conjugate             | Site-directed conjugation to ATTO 647N  |
| Excitation/ Emission  | Excitation max: 647 nm, Emission max: 669 nm  |
| DOL                   | 1 fluorophore per Nanobody  |
| Purity                | Recombinantly expressed and purified  |
| Form                  | Buffered aqueous solution   |
| Storage buffer        | 1X PBS, 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**
- 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.*
  - Wash samples three times with PBS (Phosphate Buffered Saline). Do not store fixed cells.
  - Permeabilization:** Add PBS containing 0.5% Triton X-100 to samples and incubate for 5 min at room temperature.
  - Wash samples twice with PBS.
  - Blocking:** Add 4% BSA in PBS to samples and incubate for 10 min at room temperature.
  - 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.*
  - 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.*

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