# RFP-Booster for Immunofluorescence of RFP-Fusion Proteins

For the immunofluorescence of RFP-fusion proteins in fixed cells.
*Only for research applications, not for diagnostic or therapeutic use*

## 1. Introduction

Red fluorescent proteins (RFP) and variants thereof are widely used to study protein localization and dynamics in living cells. However, photo stability and quantum efficiency of RFP are not sufficient for Super-Resolution Microscopy (e.g. 3D-SIM or STED) of fixed 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_ATTO647N, a specific RFP-binding protein coupled to the fluorescent dye ATTO 647N (from ATTO-TEC), reactivates, boosts and stabilizes your RFP signal (for a complete list of recognized RFP variants, please visit the FAQ section at www.chromotek.com).

## 2. Content

| Reagent | Code | Quantity |
|---|---|---|
| RFP-Booster_ATTO647N | rba647N-100 | 100 µl |
| RFP-Booster_ATTO647N | rba647N-10 | 10 µl |

Concentration: 0.5 g/L. Storage buffer: 1x PBS, 0.09% sodium azide.

## 3. Optical Properties

**ATTO 647N:** Excitation range 615 - 660 nm ($\lambda_{abs}$= 647 nm)
Emission range 669 - 750 nm ($\lambda_{fl}$= 669 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.
Stable for 6 month. Do not freeze. Protect from light.

## 5. 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 on the cells for 3 min, wash as in p.2 and proceed directly with p.5 of the 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.

   *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.
9. **Mounting:** Rinse sample shortly in water to prevent salt crystal formation. Mount in VectaShield (Vector Labs) or other mounting media with anti-fading agents and seal mounted coverslips with clear nail polish.

**Suggested buffer composition**

| Buffer | Composition |
|---|---|
| Fixation buffer | 3.7% formaldehyd 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

| RFP Toolbox | code |
|---|---|
| RFP-Trap®_M | rtm-20; rtm-100; rtm-200; rtm-400 |
| RFP-Trap®_M Kit | rtmk-20 |
| RFP-Trap®_A | rta-20; rta-100; rta-200; rta-400 |
| RFP-Trap®_A Kit | rtak-20 |
| Blocked agarose beads | bab-20 |
| Blocked magnetic beads | bmp-20 |
| RFP antibody | 3f5 |
| RFP antibody | 5f8 |