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_Atto594, a specific RFP-binding protein coupled to the fluorescent dye ATTO 594 (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_ATTO594	rba594	100 µg

Storage buffer: 1x PBS, 0.09% sodium azide

3. Optical properties

ATTO 594: Excitation range 560 - 615 nm (λ_{abs}= 601 nm)
Emission range 615 - 680 nm (λ_{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.
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.
2. Wash samples three times with PBS containing 0.1% Tween 20 (PBST).
3. **Permeabilization:** Add PBS containing 0.5% Triton X-100 to samples and incubate for 5 min at room temperature.
   Alternatively permeabilize cells by incubating samples in 100% methanol for 5 min at -20°C.
4. Wash samples twice with PBST.
5. **Blocking**: Add 4% BSA in PBST to samples and incubate for 10 min at room temperature.
6. **RFP-Booster incubation**: Dilute RFP-Booster 1:200 – 1:400 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 PBST.
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; 0.1% Tween 20
Blocking buffer	4% BSA (w/v); PBS; 0.1% Tween 20

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

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