GFP-Booster for Immunofluorescence of GFP-Fusion Proteins

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

1. Introduction

Green fluorescent proteins (GFP) and variants thereof are widely used to study protein localization and dynamics in living cells. However, photo stability and quantum efficiency of GFP 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 GFP signal. The GFP-Booster_ATTO488, a specific GFP-binding protein coupled to the fluorescent dye ATTO 488 (from ATTO-TEC), reactivates, boosts and stabilizes your GFP signal (for a complete list of recognized GFP variants, please visit the FAQ section at www.chromotek.com).

2. Content

Reagent	Quantity	Code
GFP-Booster_ATTO488	100 µl	gba488-100
GFP-Booster_ATTO488	10 µl	gba488-10

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

3. Optical Properties

ATTO 488: Excitation range 480 - 510 nm (λ_{abs}= 501 nm)
Emission range 520 - 560 nm (λ_{fl}= 523 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. **GFP-Booster incubation**: Dilute GFP-Booster 1:200 in blocking buffer and incubate for 1 h at room temperature.

   Note: For multiplexing protocols, you can combine GFP-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 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

GFP Toolbox	code
GFP-Trap®_M	gtm-20; gtm-100; gtm-200; gtm-400
GFP-Trap®_M Kit	gtmk-20
GFP-Trap®_A	gta-20; gta-100; gta-200; gta-400
GFP-Trap®_A Kit	gtak-20
GFP-multiTrap	gtp-96; gtp-480
Blocked agarose beads	bab-20
Blocked magnetic beads	bmp-20
GFP antibody	3h9