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GFP-Booster for Immunofluorescence of GFP-Fusion Proteins

For the immunofluorescence staining of GFP-fusion proteins in fixed cell.

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, the photo stability and the quantum efficiency of GFP is not sufficient for Super-Resolution Microscopy (e.g. 3D-SIM or STED) of fixed samples from cells expressing GFP-fusion proteins to visualize specific structures. Furthermore, many cell biological methods such as HCl treatment for BrdU-detection, the EdU-Click-iT™ treatment or heat denaturation for FISH lead to disruption of GFP signal.

GFP-Booster specifically highlights GFP (eGFP) and yellow fluorescent protein derivatives (YFP, eYFP and Venus)

Use GFP-Booster _Atto488, a specific GFP-binding protein coupled to the fluorescent dye ATTO 488 (from ATTO-TEC) to reactivate, boost and stabilize GFP signal.

2. Content

GFP-Booster_Atto488

Catalog no.: gba488 / 100µg

3. Stability and Storage

Store material at 4°C, do not freeze. Protect from light.

4. Optical Properties

ATTO 488: Fluorescence is excited most efficiently in the range 480 - 510 nm. For instance a 488 nm laser is very suitable for excitation (λ_{abs}= 501 nm). Fluorescence is emitted most efficiently in the range 520 - 560 nm (λ_{fl}= 523 nm).

For further information please refer to http://www.atto-tec.com

5. Suggested Protocol

1. **Fixation**: 4% paraformaldehyde (PFA) or 1:10 formalin (37% formaldehyde, 10-15% MetOH) in PBS, 10 min., RT.
2. Wash 3x with PBS containing 0.1% Tween 20 (PBST). **Critical**: do not let coverslips “dry”.
3. **Permeabilisation:** PBS containing 0.5% Triton X-100, 5 min., RT.
   Alternatively permeabilise by incubating in 100% methanol for 5min at -20°C.
4. Wash 2x with PBST.
5. **Blocking**: 4% BSA in PBST, 10 min, RT.
6. **GFP-Booster incubation**: dilute GFP-Booster 1:200 in blocking buffer and incubate 1 h, RT.
   Note: For multiplexing protocols you can combine GFP-Booster with any other antibody.
7. Wash 3x 5-10 min in PBST.
8. If required counterstain with DNA fluorescent dyes, e.g. DAPI.
9. Before mounting coverslips can be very briefly rinsed in water to prevent salt crystals to form.
10. Mount in VectaShield (Vector Labs) or other mounting media with anti-fading agents and seal mounted coverslips with clear nail polish.

Please note: Optimal dilutions/ concentrations should be determined by the end user

6. Notes

- GFP-Booster can be combined with conventional primary and secondary antibody staining
- Use before GFP-Booster prior to GFP-denaturing steps