# GFP-Booster for Immunofluorescence Detection of GFP-Fusion Proteins

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

## 1. Product

The GFP-Booster Alexa Fluor® 647 is an anti-GFP Nanobody coupled to Alexa Fluor® 647.

## 2. Introduction

Green fluorescent protein (GFP) and its variants are widely used to study protein localization and dynamics in cells. However, photo-stability and quantum efficiency of GFP 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 GFP signal. The GFP-Booster reactivates, enhances, and stabilizes the GFP-signal.

*Note: This product is an improved version of products gbAF647 and gba647n.*

## 3. Properties

| | |
|---|---|
| Product size | gb2AF647-10: 10 µL<br>gb2AF647-50: 50 µL |
| Format | Alpaca single domain antibody, Nanobody or $V_HH$; monovalent |
| Target/ Specificity | GFP and GFP variants. See www.chromotek.com for a list of recognized GFP variants. |
| Conjugate | Site-directed conjugation to Alexa Fluor 647 |
| Excitation/ Emission | Excitation max: 650 nm, Emission max: 665 nm |
| DOL | 2 fluorophores per Nanobody |
| Purity | Recombinantly expressed and purified |
| Form | Buffered aqueous solution |
| Storage buffer | 10 mM HEPES pH 7.0, 500 mM NaCl, 5 mM EDTA,<br>Preservative: 0.09% Sodium azide, Safety datasheet (SDS): **Sodium azide SDS** |
| 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

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 to cells for 3 min, wash as in step 2 and proceed directly with step 5 of this 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 1:500 – 1:1,000 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 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 formation of salt crystals. 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 |
|---|---|
| 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 |

## 5. Support/ Troubleshooting

Please refer to our FAQ section at www.chromotek.com or contact *support@chromotek.com*

*Only for research applications, not for diagnostic or therapeutic use.*