MASSIVE PHOTONICS

DNA-PAINT KIT

MASSIVE-TAG-Q anti-GFP

Expiration after 6 months *(For research use only)*

CONTENT

SINGLE DOMAIN ANTIBODY

- **FluoTagQ® anti-GFP** (clone: 1H1) **+ Docking site 3** (To be measured with Imager 3)
- Concentration: 5 µM Protein, 5 µM DNA (1 DNA strand per protein)
- Volume: 100 µL
- Storage buffer: PBS, 50 % glycerol + 0.05 % NaN_3
- Storage: -20 °C
- Recommended dilution: 1:200 – 1:500 (for optimal results the dilution needs to be optimized depending on the target accessibility and expression level)

IMAGERS

- **Imager 3** Cy3B, ATTO 565 or ATTO 655
- Concentration: 1 µM in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- Volume: 300 µL
- Storage: -20 °C

  Note: We recommend preparing intermediate dilutions (e.g. 500 nM – 1 µM) in TE buffer or ultrapure water and store them at -20 °C (stable for several freeze and thaw cycles). Further dilutions should be prepared fresh before use. Low imager concentrations are not stable in plastic tubes.

BUFFERS

- **Antibody incubation buffer**, 50 mL, store at 2-8 °C

  Note: For longer-term storage we recommend to store aliquots at -20 °C.
- **Washing buffer (10×)**, 20 mL, store at room temperature (to be diluted 1:10 in water before use)
- **Imaging buffer**, 50 mL, store at room temperature

SAMPLE PREP. PROTOCOL

1. Prepare sample using a protocol optimized for your GFP-tagged protein target.
2. Block cells in antibody incubation buffer for ~30 minutes.
3. Dilute sdAbs in antibody incubation buffer.
4. Incubate for 1 hour at room temperature or overnight at 4 °C.
5. Wash three times with washing buffer (1×).
6. Optional: Incubate fiducial markers.
7. Wash once with imaging buffer before adding the final imaging solution with imager strands.
8. Before imaging: Add imager strands diluted in imaging buffer. We recommend a starting concentration of 1 nM. However, the optimum imager concentration strongly depends on the target and labeling density. Thus, the imager concentration should be adjusted such that distinct single molecule blinking events can be observed
9. After imaging, exchange buffer to washing buffer (1×) for storage.

IMAGING PARAMETERS

- Exposure time: 100-200 ms
- Laser-Intensity: ~200 W/cm² (561 nm) and ~300 W/cm² (640 nm). This intensity might vary due to different illumination depths/modes (TIRF/HILO). For dense targets we recommend increasing the laser power to enhance blinking.
- Total imaging time/target: 30 min