MASSIVE PHOTONICS

DNA-PAINT KIT

MASSIVE-RESI anti-GFP

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

CONTENT

SINGLE DOMAIN ANTIBODY

- **FluoTagQ® anti-GFP** (clone: 1H1) **+ Docking site F1, F2. F3, or F4** (To be measured with Imager F1, F2, F3, or F4)
  This kit stains GFP-tagged proteins with a mixture of sdABs conjugated to orthogonal docking strands for RESI (Resolution Enhancement by Sequential Imaging)
- 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 F1, F2, F3, and F4** Cy3B
- Concentration: 1 µM in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- Volume: 300 µL
- Storage: -20 °C (1 µM imager solutions are stable for multiple freeze-and-thaw cycles)
  Optional: Prepare 50 µL aliquots and store at -20 °C. Working aliquots can be stored at 4 °C for short-term or -20 °C for long term
  Note: 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.
5. Wash three times with washing buffer (1×).
6. Incubate fiducial markers (gold nanoparticles).
7. Wash once with imaging buffer before adding the first RESI imaging solution containing imager strands.
8. Before imaging: Add imager strands diluted in imaging buffer to determine the ideal imaging concentration. 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. Add imaging buffer containing a RESI imager and image the first RESI round. Note: you should observe less blinking than in a normal DNA-PAINT imaging experiment.
10. After imaging, wash the sample 3x with Washing Buffer, or until no further blinking is observed.
11. Add imager strands diluted in imaging buffer for the next RESI round.
12. Repeat steps 9 – 11 until all four RESI rounds have been completed.
13. Wash with washing buffer and store your sample.

IMAGING PARAMETERS

- Exposure time: 50 - 100 ms
- Laser-Intensity: ~200 W/cm² (561 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 per RESI round: ~15 - 30 min (\Temperature: The kit is optimized for image acquisition at 21 - 25 °C. At higher temperatures shorter exposure times and higher laser powers are required.

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