

DNA-PAINT KIT

MASSIVE-sdAB-FAST anti-CTLA4

Expiration after 6 months

(For research use only)

CONTENT

SINGLE DOMAIN ANTIBODY

- **sdAB anti-CTLA4** (clone: 1G3)* + **FAST docking site F2** (To be measured with Imager F2 or F2-LB or F3-FG)
- Concentration: 5 μ M Protein, 5 μ M DNA (1 DNA strand per protein)
- Volume: 100 μ L
- Storage buffer: PBS, 50 % glycerol + 0.05 % NaN_3
- Storage: -20 °C
- Recommended dilution: 1:100-1:500 (for optimal results the dilution needs to be optimized depending on the target accessibility and expression level)

IMAGERS

- **Imager F2** or **F2-LB** Cy3B or ATTO 655 or **F2-FG** Cy3B
 - Concentration: 1 μ M in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
 - Volume: 300 μ L
 - Storage: -20 °C (1 μ M imager solutions are stable for multiple freeze-and-thaw cycles)
- Optional: Prepare 50 μ L 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**, 30 mL, store at 2-8 °C
- Note: For longer-term storage we recommend to store aliquots at -20 °C.
- **Washing buffer (10 $\times$)**, 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 targets.
2. Block cells in Antibody incubation buffer for ~30 minutes.
3. Dilute sdABs in Antibody incubation buffer.
4. Incubate for 30-60 min at room temperature.
5. Wash three times with washing buffer (1 $\times$).
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 $\times$) for storage at 4 °C.

IMAGING PARAMETERS

- Exposure time: FAST: 30-75 ms; FAST-LB: 100-150 ms; FAST-FG: 75-150 ms
- Laser-Intensity: FAST: ~300 W/cm² (561 nm) and ~500 W/cm² (640 nm); FAST-LB: ~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: FAST: 5-15 min; FAST-LB: 30 min; FAST-FG: 15-60 min (Depends on target density and applied imager concentration).
- Temperature: The kit is optimized for image acquisition at 21-25 °C. At higher temperatures shorter exposure times and higher laser powers are required.

* sdABs developed and manufactured by NanoTag Biotechnologies are sold under license. Unmodified and fluorophore-coupled sdABs (FluoTag®) remain available for direct purchase from NanoTag Biotechnologies.

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