DNA-PAINT KIT

MASSIVE-AB 2-PLEX

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

CONTENT

SECONDARY ANTIBODIES

- Polyclonal Donkey **Anti-Mouse IgG + Docking site 1** (To be measured with Imager 1)
- Polyclonal Donkey **Anti-Rabbit IgG** + **Docking site 2** (To be measured with Imager 2)
- Volume: 100 µL
- Storage: 2-8 °C
- Storage buffer: PBS + 0.05 % NaN_3
- 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 1** Cy3B, ATTO 565 or ATTO 655
- **Imager 2** 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.

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×)**, 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 target and primary antibody staining.
2. Wash with washing buffer (1×).
3. Dilute secondary DNA-PAINT antibodies in antibody incubation buffer.
4. Incubate for 1 hour at room temperature.
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: ~250 W/cm² (561 nm) and ~500 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