

DNA-PAINT KIT

MASSIVE-AB 3-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)
- Polyclonal Donkey Anti-Rat IgG + Docking site 3 (To be measured with Imager 3)
- Volume: 100 μL
- · Concentration: 0.5 mg/mL
- Storage: 2 8 °C
- Storage buffer: PBS + 0.05 % NaN₃
- 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 or ATTO 655
- Imager 2 Cy3B or ATTO 655
- Imager 3 Cy3B or ATTO 655
- Concentration: 1 μM in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- Volume: 300 μL
- Storage: 4 °C (1-2 weeks), -20 °C (long-term), stable for multiple freeze-and-thaw cycles.
 Optional: Prepare 50 µL aliquots and store at -20 °C. Working aliquot 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 table 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 $^{\circ}\text{C}.$
- Washing buffer (10x), 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 (1x) for storage.

IMAGING PARAMETERS

- Exposure time: 100 150 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
- Temperature: The kit is optimized for image acquisition at 21 25 °C. At higher temperatures shorter exposure times and higher laser powers might be required.



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