MASSIVE PHOTONICS

DNA-PAINT KIT

MASSIVE-AB-FAST anti-Bassoon

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

CONTENT

SINGLE DOMAIN ANTIBODY

- **Mouse anti-Bassoon IgG2a kappa light chain with Anti-Mouse IgG2a/b** (clone: 14A4) **+ FAST docking site F4** (To be measured with Imager F4 or F4-LB)
- Concentration: 1.3 µM of antibody preincubated with sdAB
- Volume: 100 µL
- Storage buffer: PBS, 40 % glycerol + 0.04 % NaN_3
- Storage: 2-8 °C
- Recommended dilution: 1:20-1:100 (for optimal results the dilution needs to be optimized depending on the target accessibility and expression level)

IMAGERS

- **Imager F4 or F4-LB** Cy3B or ATTO 655
- 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**, 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 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×).
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 at 4 °C.

IMAGING PARAMETERS

- Exposure time: FAST: 30-50 ms; FAST-LB: 100-150 ms
- Laser-Intensity: ~300 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: 5-15 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.

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