

## DNA-PAINT KIT

# MASSIVE-Actin

Expiration after 6 months

(For research use only)

### CONTENT

#### AFFINITY REAGENT

- **MASSIVE-Actin**
- Concentration: 10  $\mu$ M
- Volume: 100  $\mu$ L
- Storage: -20 °C
- Storage buffer: PBS + 0.05 % NaNa<sub>3</sub>
- Recommended dilution: 1:25,000 - 1:50,000 (for optimal results the dilution needs to be optimized depending on the target accessibility and expression level)

#### IMAGERS

- No imagers are needed or present in this kit. MASSIVE-Actin-Cy3B binds transiently to F-actin to create single-molecule blinking.

#### BUFFERS

- **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. Fix sample using 4% Paraformaldehyde for 15 minutes.
2. Optional: Prepare sample using a protocol optimized for your target and immunostaining strategy.
3. Wash three times with washing buffer (1 $\times$ ).
4. Optional: Incubate fiducial markers.
5. Wash once with imaging buffer before adding the final imaging solution containing MASSIVE-Actin-Cy3B.
6. Before imaging: Add MASSIVE-Actin-Cy3B diluted in imaging buffer. We recommend a starting concentration of 100-200 pM. However, the optimum concentration strongly depends on the sample and its F-actin density. Thus, MASSIVE-Actin-Cy3B concentration should be adjusted such that distinct single molecule blinking events can be observed. Wash with imaging buffer before adding the final imaging solution with imager strands.
7. After imaging, exchange buffer to washing buffer (1 $\times$ ) for storage.

### IMAGING PARAMETERS

- Exposure time: 50 ms
- Laser-Intensity:  $\sim$ 300 W/cm<sup>2</sup> (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/target: 30 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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