DNA-PAINT KIT

MASSIVE-RNA-FISH TFRC

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

CONTENT

RNA-FISH PROBES

- FISH Probe Set for TFRC (NM_001128148.3) with FAST docking site F2 (To be measured with Imager F2-FG)
- DNA Concentration: 10 µM
- Volume: 100 µL
- Storage: -20 °C

IMAGERS

- Imager F2-FG Cy3B
- 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 2-8 °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.

BUFFER

- RNA Imaging Buffer, 50 mL, store at 2-8 °C

Required: Massive-RNA-FISH Buffer Kit (not included)

SAMPLE PREP. PROTOCOL

THIS PROTOCOL IS OPTIMIZED FOR CULTURED CELLS

1. Cell Fixation:
   - Fix cells with the *Fixation solution* for 15 minutes at room temperature.
   - Wash samples with *Washing Buffer A*
2. Permeabilization:
   - Incubate the sample in the *Permeabilization Solution* for 15 minutes at room temperature.
3. Pre-Hybridization:
   - Wash sample in *Washing Buffer A* for 5 minutes at room temperature.
   - Incubate the sample in *Washing Buffer B* for 30 minutes at room temperature
4. Hybridization:
   - Dilute the FISH Probes in a 1:100 ratio in *Hybridization Buffer*.
   - Add the resulting Hybridization solution to the sample and incubate overnight at 37 °C in a humidified chamber.
5. Post-Hybridization Washes:
   - Wash the samples three times with *Washing Buffer B*.
   - Each wash should last for 15 minutes at 37 °C.

Further steps - see next page

6. Imaging Preparation:
   - Wash once with *RNA Imaging Buffer* to prepare the sample for imaging.
   - Dilute the Imager strands to a desired final concentration in *RNA Imaging Buffer* and apply to the sample immediately before imaging.

     Note: The optimal imager concentration can vary depending on the labeling density. A starting concentration of 1 nM Imager is adequate. Adjust as needed to observe distinct single-molecule blinking events.
7. Post-Imaging Storage:
   - After imaging, exchange the buffer to *Washing Buffer A* for storage at 2-8 °C.

IMAGING PARAMETERS

- Microscopy Mode: TIRF / HILO / EPI
- Exposure Time: 75-150 ms
- Laser Power: ~30 mW (561 nm). This may require optimization depending on your specific setup and illumination mode.
- Total Frames: Acquire 10,000 - 20,000 frames per target area.
- Temperature: The kit is optimized for image acquisition at 21–25 °C.

ADDITIONAL INFORMATION

- Optional: To minimize the risk of RNA degradation by RNAse and measurement artifacts, RNase inhibitors may be included in all working buffers. This is particularly advisable if contamination or unexpected signals are encountered during measurements.
- Recommended RNAse Inhibitor: Ribonucleoside Vanadyl Complex diluted in buffers to a final concentration of 2 mM

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