

DNA-PAINT KIT**MASSIVE-RNA-FISH BUFFER KIT**

Expiration after 6 months

(For research use only)

CONTENT**BUFFERS**

- Fixation Solution , 25 mL, store at room temperature
- Washing Buffer A , 100 mL, store at room temperature
- Permeabilization Solution, 25 mL, store at room temperature
- Washing Buffer B , 100 mL, store at 2-8 °C
add 10 % deionized formamide to the required buffer volume that is used for the subsequent experiment
- Hybridization Buffer, 100 mL, store at 2-8 °C
add 10 % deionized formamide and 2 mM RNase inhibitor to the required buffer volume that is used for the subsequent experiment
- RNA Imaging Buffer, 50 mL, store at 2-8 °C

**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.
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.
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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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