

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

Expiration after 6 months

(For research use only)

**CONTENT****RNA-FISH PROBES**

- FISH Probe Set for GAPDH (NM\_002046.7) with FAST docking site F1 (To be measured with Imager F1-FG)
- DNA Concentration: 10  $\mu$ M
- Volume: 100  $\mu$ L
- Storage: -20 °C

**IMAGERS**

- Imager F1-FG Cy3B
- Concentration: 1  $\mu$ M in TE buffer (10 mM Tris, 1 mM EDTA, pH 8)
- Volume: 300  $\mu$ L
- Storage: -20 °C (1  $\mu$ M imager solutions are stable for multiple freeze-and-thaw cycles)  
*Optional: Prepare 50  $\mu$ L 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

# GENERAL TERMS AND CONDITIONS

These Terms and Conditions for Sales of Products constitute a binding legal agreement between Massive Photonics GmbH ("Massive") and the customer or authorized distributor ("Purchaser") refers to either the customer and/or authorized distributor unless alternative terms are set forth in a signed written agreement between Massive and the Purchaser.

- 1. Payment.** Net amount of invoice is due within 30 days of invoice date unless the invoice states otherwise. Massive may require payment in advance if justified in Massive's opinion by Purchaser's financial condition or payment history.
- 2. Shipment.** Unless specified by Purchaser, Massive will select a carrier of its choice. Purchaser will be responsible for costs of transportation, shipping and handling, and applicable importation fees. Shipments are insured at Purchaser's expense and made at Purchaser's risk. Massive may make partial shipments. Shipment dates are estimates only.
- 3. Taxes.** All taxes will be added to product price and will be paid by Purchaser, unless Purchaser provides Massive with a valid tax exemption certificate.
- 4. Delays.** Massive will not be liable for loss or damage of any kind resulting from delay or failure in fulfilling orders, if such delay or failure is directly or indirectly caused by factors beyond Massive's reasonable control.
- 5. Limited Warranties and Remedies.** Massive warrants that products are free from defects in material and manufacture. Should any product prove defective, Purchaser must notify Massive promptly, no later than sixty (60) days from the date of the delivery, stating full particulars in support of Purchaser's claim. Massive will replace defective products or components, or apply an appropriate credit, upon Massive's investigation as may be necessary under the circumstances. The foregoing is Massive's sole obligation, and Purchaser's sole remedy, in the event of a claimed defect. UNDER NO CIRCUMSTANCES SHALL MASSIVE BE LIABLE FOR SPECIAL, INDIRECT, CONSEQUENTIAL OR OTHER DAMAGES, LOSSES, OR EXPENSES IN CONNECTION WITH THE USE OF OR INABILITY TO USE PRODUCTS FOR ANY PURPOSE; NOR SHALL MASSIVE'S LIABILITY IN ANY EVENT EXCEED THE PURCHASE PRICE PAID FOR THE PRODUCTS INVOLVED. The advice of Massive's technical staff may be made available to Purchaser; however, because Massive does not control or supervise use of products after sale, Massive does not warrant or guarantee such advice. NO WARRANTY OR GUARANTEE, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY AS TO MERCHANTABILITY OR FITNESS FOR ANY PURPOSE, IS MADE EXCEPT AS SET FORTH HEREIN, AND MASSIVE EXPRESSLY DISCLAIMS ALL OTHER WARRANTIES.
- 6. Cancellation and Changes.** Except as otherwise expressly provided in Section 4, above, an order once placed and accepted by Massive's confirmation is non-cancellable, unless Massive consents to such cancellation in writing and Purchaser pays any applicable cancellation charges.
- 7. Research Use Only.** Purchaser acknowledges that products are for research use only; not for diagnostic use. Products have not been subjected to regulatory review or cleared by the United States Food and Drug Administration ("FDA"). Purchaser agrees not to use any product: (a) in any manner requiring FDA or other regulatory approval or registration, such as uses in diagnostic or therapeutic procedures; (b) for manufacturing; or (c) for quality control related to (a) or (b). The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration.
- 8. Proper Use.** Purchaser agrees (a) not to use any product for a purpose other than its intended use; (b) to store products according to Massive storage requirements; (c) to use products only as specified in the applicable written instructions provided by Massive; and (d) to use products only for Purchaser's internal research use, and not for resale or commercial distribution. If Purchaser fails to comply with any of these term and conditions, Massive will be released from its performance and liability obligations to Purchaser and Purchaser will pay all costs or damages, including legal fees, incurred by Massive, caused by such failure to comply.
- 9. No Reverse Engineering.** Purchaser agrees not to, nor allow any third party to, reverse engineer or otherwise attempt to discover the identity of a composition, nucleic acid sequence, or other component of a product.
- 10. Governing Law.** These terms and conditions shall be governed and construed under the laws of the Federal Republic of Germany without reference to principles of conflicts of law. Any action concerning this sale must be filed in the courts where Massive has its place of business and each party submits to the exclusive jurisdiction of these courts.