**Product Information**

**PMA™ dye, 20 mM in H₂O**

Catalog Number: 40019

Size: 100 uL

Molecular Information:

MW: 511

Color and Form: Orange red liquid

Spectral Property: λ<sub>abs</sub> = 464 nm (before photolysis); λ<sub>abs</sub>/λ<sub>em</sub> = ~510/~610 nm (following photolysis and covalent attachment to DNA/RNA)

Storage and Handling

PMA, 20 mM in H₂O should be stored at -20 °C protected from light. When stored as recommended the dye is stable for at least six months from date of receipt.

**Product Description**

PMA is a high affinity photoreactive DNA binding dye developed by Biotium. The dye is weakly fluorescent by itself but becomes highly fluorescent upon binding to nucleic acids. It preferentially binds to dsDNA with high affinity. Upon photolysis, the photoreactive azido group on the dye is converted to a highly reactive nitrene radical, which readily reacts with any hydrocarbon moiety at the binding site to form a stable covalent nitrogen-carbon bond, thus resulting in permanent DNA modification (Figure 1). The dye is cell membrane-impermeable and thus can be selectively used to modify only DNA from dead cells with compromised membrane integrity, while leaving DNA from viable cells intact. PMA inhibits PCR amplification of modified DNA templates, making the dye useful in the selective detection of viable pathogenic cells by quantitative real-time PCR (Figure 2).

![Figure 1. Mechanism of nucleic acid modification by PMA.](image1.png)

**Light sources for photoactivation**

A halogen light source ≥ 600 W can be used for light-induced cross-linking of PMA to DNA. We use a Commercial Electric LS300 HD lamp with two 300 W halogen bulbs. Alternatively, a thermally-stable blue LED light source such as the PhAST Blue Photoactivation System for Tubes (available from GenIUL or Neutec Group) or the LED Active Blue instrument (IB Applied Science) may be used according to the manufacturer’s instructions.

![Figure 2. Principle of PMA modification for quantitation of viable bacteria by qPCR.](image2.png)

**Protocol for treating bacteria with PMA for qPCR**

The following is a protocol for treating cultured laboratory strains of bacteria with PMA. Treatment of complex biological or environmental samples such as feces or soil may require optimization of sample dilution for PMA and light treatment.

1. Inoculate an appropriate media broth with bacteria (volume is dependent on size of experiment).
2. Shake cultures at 200 RPM at 37°C overnight.
3. Continuing culturing bacteria until the OD<sub>600</sub> of the culture is approximately 1.
4. For positive control samples, heat inactivate bacteria at 100°C for 10 min. To confirm heat inactivation of bacteria, plate 250 uL of control and heat inactivated bacteria on the appropriate media plate. Seal the plate with Parafilm and place at 37°C. Check for colony growth at 24 hours, and again after 3-6 days.
5. Pipet 500 uL aliquots of bacterial culture into clear microcentrifuge tubes.
6. Add the appropriate volume of PMA stock for a final concentration of 50 uM (e.g., 1.25 uL of 20 mM stock in 500 uL).
7. Incubate tubes in the dark for 5 minutes at room temperature. Flick tubes occasionally to mix, or incubate on a rocker covered with aluminum foil.
8. Expose samples to light to cross-link PMA to DNA. See information on light sources at the top of this column.
   a. For light exposure using a halogen light source: lay tubes on a block of ice set 20 cm from the light source. The ice block should be in a clear tray, set on a shaker or rocking platform to ensure continuous mixing during light exposure. Set the lamp so that the light source is pointing directly downward onto the samples (up to 45° downward slant is OK). Place a piece of aluminum foil under the clear tray to reflect the light upward toward the bottom of the tubes. Expose samples to light for 5 min.
   b. For blue LED photoactivation systems, follow the manufacturer’s instructions for light exposure of samples.
9. Pellet cells by centrifuging at 5,000 x g for 10 minutes. If no pellet is visible, centrifuge again at maximum speed for 5 minutes.
10. Extract genomic DNA for qPCR analysis using a standard protocol or commercially available kit. Use an appropriate protocol or kit for DNA extraction from complex biological or environmental samples (e.g., feces or soil).
11. Perform qPCR using primers against an appropriate genomic DNA target for your organism of interest. DNA templates modified with PMA will show delayed amplification by qPCR (Figure 3).

![Figure 3. Difference in Ct value with and without PMA treatment.](image3.png)
Biotium offers a broad selection of novel fluorescence reagents for molecular and cellular biology. Please visit www.biotium.com for more information.

### Related products

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<tr>
<th>Product</th>
<th>Size</th>
<th>Cat. No.</th>
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<tr>
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<td>1 mg</td>
<td>40013</td>
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<tr>
<td>EvaGreen® Dye, 20X in water (trial size)</td>
<td>1 mL</td>
<td>31000-T</td>
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<tr>
<td>Fast Plus EvaGreen® qPCR Master Mix (trial size)</td>
<td>100 rxn</td>
<td>31020-T</td>
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<td>GelRed™ Nucleic Acid Gel Stain, 10,00X in H₂O</td>
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<td>GelGreen™ Nucleic Acid Gel Stain, 10,00X in H₂O</td>
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<td>Live Bacterial Gram Stain Kit</td>
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<td>Bacillary Viability and Gram Stain Kit</td>
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### Selected References


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Figure 3. Effect of PMA on qPCR of DNA from live and heat-inactivated E. coli. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA-treated live and heat-killed E. coli. (B) The ΔCt of live and killed E. coli with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA).

Figure 4. Effect of PMA and light exposure on qPCR of DNA from live and heat-killed S. epidermidis (Staph) DNA. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). Samples were exposed to light for 5 minutes after PMA treatment as indicated. qPCR was performed using primers against a region of the 16S rRNA gene.

Figure 5. ΔCt of live, heat-killed, and mixed live/killed E. coli and S. epidermidis (Staph) with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding PMA-treated sample (Ct with PMA – Ct without PMA). qPCR was performed using primers against a region of the 16S rRNA gene.