PMA™ Enhancer for Gram Negative Bacteria, 5X Solution

Catalog Number: 31038

Unit size: 16 mL

Storage and Handling
PMA Enhancer should be stored at 4 °C. When stored as recommended, the product is stable for at least six months from date of receipt.

Color and Form: Clear liquid

Product Description
PMA Enhancer for Gram Negative Bacteria is designed for use with PMA, a nucleic-acid modifying dye, to selectively detect viable gram-negative bacteria using real-time PCR.

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. The dye is cell membrane-impermeable and thus can be used to selectively modify DNA from dead cells with compromised membrane integrity, while leaving DNA from viable cells intact. PMA inhibits PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases (Nocker et al. 2006). Consequently the dye is useful in the selective detection of viable cells by quantitative real-time PCR (Figure 1).

When PMA Enhancer is added to gram-negative bacteria before treatment with PMA, dead cell DNA levels are further decreased, and thus live/dead cell discrimination is improved. The amount of improvement varies depending on such factors as the bacterial strain and the way that the bacterial were killed. Enhancer gives the most improvement when bacteria are dead but their membranes are not completely disrupted, as occurs after mild heat treatment.

Note 1: The Enhancer should not be used with gram positive bacteria, where it may cause a loss of live cell DNA. If you plan to detect both gram-negative and gram-positive bacteria, you should use PMA alone without Enhancer.

Protocol for treating gram-negative bacteria with PMA plus Enhancer for qPCR
The following is a protocol for treating cultured laboratory strains of gram-negative 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. PMA Enhancer improves the activity of PMA on gram-negative bacteria, but has a detrimental effect on gram-positive bacteria. If both gram-negative and gram-positive bacteria are to be treated in one sample, Enhancer should not be used.

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 cultivating bacteria until the OD of the culture is approximately 1.
4. For dead cell control samples, heat inactivate bacteria at 58°C for 3 hours. To confirm killing of bacteria, plate 10 uL of heat inactivated bacteria on the appropriate media plate, and 10 uL of a 1:100 dilution of control bacteria on another plate. Place the plate at 37°C and check for colony growth after 24-48 hours.
5. Pipette 400 uL aliquots of bacterial culture into clear microcentrifuge tubes.
6. Add 100 uL of 5X Enhancer to each tube, for a 1X final Enhancer concentration.
7. Working quickly and in low light, prepare a working stock of PMA by diluting to 5 mM in water. Add 2.5 uL of working stock to each tube for a final concentration of 25 uM.
8. Incubate tubes in the dark for 10 minutes at room temperature. Flick tubes occasionally to mix, or incubate on a rocker covered with aluminum foil.
9. Expose samples to light to cross-link PMA to DNA. See information on light sources below.
   a. For best results, we recommend that the photo-crosslinking be carried out on Biotium’s PMA-Lite LED Photolysis Device. 15 min exposure should be sufficient for complete PMA activation.
   b. Commercial halogen lamps (>600 W) for home use have been employed for photoactivating PMA in some publications, though results have not been consistent due to inevitable variation in the set-up configurations. If you decide to use a halogen lamp, we recommend that you lay tubes on a block of ice set 20 cm from the light source. The ice block should be in a clear tray with a piece of aluminum foil under the clear tray to reflect the light upward. Set the lamp so that the light source is pointing directly downward onto the samples. Expose samples to light for 5-15 min.
10. Pellet cells by centrifuging at 5,000 x g for 10 minutes.
11. Extract genomic DNA 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).
12. 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, and a further delay when Enhancer is used (Figure 2).

Note 2: Amplicons as short as 100 bp can be used, but longer target amplicons have been shown to decrease the signal from heat-killed PMA-treated cells (see papers from Martin et al., Banihashemi et al., and Contreras et al. under Selected References).

Note 3: Part of the proposed mechanism of action of PMA is the removal of PMA-bound DNA from samples via precipitation; therefore the amount of input DNA in each sample should not be normalized between samples. Instead, PCR should be performed using equal volumes of gDNA eluate from each sample. For a positive control, 1 ng of live cell gDNA per reaction should be sufficient for achieving good signal. For gDNA extracted from bacterial cultures using a commercial extraction kit, 1-2 uL of eluted DNA can be used as a starting point for optimization.

Figure 1. Principle of PMA modification for quantitation of viable bacteria by qPCR. The cell membrane-impermeable PMA dye selectively and covalently modifies DNA from dead bacteria with compromised membranes. Subsequent PCR amplification of PMA-modified DNA templates is inhibited, allowing selective quantitation of viable bacteria.
**Related products**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>40015</td>
<td>EMA (ethidium monoazide)</td>
<td>5 mg</td>
</tr>
<tr>
<td>40013</td>
<td>PMA™ (propidium monoazide)</td>
<td>1 mg</td>
</tr>
<tr>
<td>40019</td>
<td>PMA™ (propidium monoazide, 20 mM in H_2O)</td>
<td>100 µL</td>
</tr>
<tr>
<td>E90002</td>
<td>PMA-Lite™ LED Photolysis Device</td>
<td>1 device</td>
</tr>
<tr>
<td>31033</td>
<td>PMA-PCR bacterial viability kit, Salmonella</td>
<td>200 assays</td>
</tr>
<tr>
<td>31034</td>
<td>PMA-PCR bacterial viability kit, M. tuberculosis</td>
<td>200 assays</td>
</tr>
<tr>
<td>31035</td>
<td>PMA-PCR bacterial viability kit, Staph. aureus</td>
<td>200 assays</td>
</tr>
<tr>
<td>31036</td>
<td>PMA-PCR bacterial viability kit, MRSA</td>
<td>200 assays</td>
</tr>
<tr>
<td>31037</td>
<td>PMA-PCR bacterial viability kit, E. coli O157:H7</td>
<td>200 assays</td>
</tr>
<tr>
<td>31050</td>
<td>PMA Real-Time PCR Bacterial Viability Kit, E. coli</td>
<td>200 assays</td>
</tr>
<tr>
<td>31051</td>
<td>PMA Real-Time PCR Bacterial Viability Kit, Listeria monocytogenes</td>
<td>200 assays</td>
</tr>
<tr>
<td>31003</td>
<td>Fast EvaGreen® qPCR Master Mix (200 µL)</td>
<td>2 x 1 mL</td>
</tr>
<tr>
<td>32000-1</td>
<td>Live Bacterial Gram Stain Kit</td>
<td>200 assays</td>
</tr>
<tr>
<td>32001</td>
<td>Bacterial Viability and Gram Stain Kit</td>
<td>200 assays</td>
</tr>
<tr>
<td>30027</td>
<td>Viability/Cytotoxicity Assay Kit for Bacterial Live and Dead Cells</td>
<td>100-1000 assays</td>
</tr>
</tbody>
</table>

Biotium offers a broad selection of novel fluorescence reagents for molecular and cellular biology. Please visit www.biotium.com for more information.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use. Biotium is not liable for any damage resulting from handling or contact with this product.

**Light sources for photoactivation**

Biotium offers the PMA-Lite™ LED Photolysis Device for light-induced cross-linking of PMA to dsDNA. The PMA-Lite™ LED Photolysis Device is a thermally-stable blue LED light source that provides even illumination to all samples. It contains a cooling unit to prevent sample overheating as well as several timer settings to allow for precisely timed light treatment.

**Selected References**


