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Product Information

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 culturing bacteria until the OD_{600} 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.

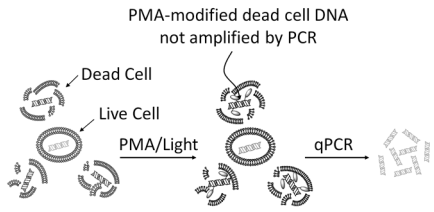

Figure 1. Principle of PMA modification for quantitation of viable bacteria by qPCR. The cell membrane-impermeable PMA dye selectively and covalently modifies DNA from dead bacteria with compromised membranes. Subsequent PCR amplification of PMA-modified DNA templates is inhibited, allowing selective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

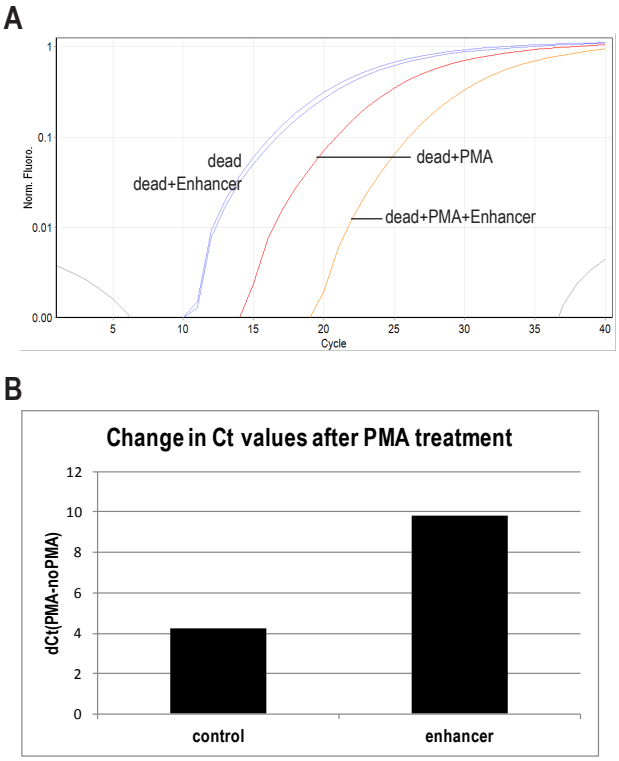

Figure 2. PMA plus Enhancer for quantitation of viable bacteria by Real-time PCR.
A. Mildly heat-killed *E. coli* were treated with PMA and/or Enhancer, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 377-bp fragment of *E. coli* DNA. Dead cells treated with PMA+Enhancer showed a significant further delay in Ct compared to dead cells treated with PMA alone.
B. For either control- or enhancer-treated cells, dCt values were calculated by subtracting the Ct without PMA from the Ct with PMA. For dead cells, the use of Enhancer increased the dCt from 4 to 10, greatly increasing the specificity of viability PMA-PCR.

Selected References

Banihashemi, A, et al. Long-amplicon propidium monoazide-PCR enumeration assay to detect viable *Campylobactor* and *Salmonella*. J. Appl Microbiol. 113(4), 863-73. (2012)

Chen, S., et al. Rapid Detection of Viable Salmonella in Produce by Coupling Propidium Monoazide with Loop-Mediated Isothermal Amplification (PMA-LAMP). Appl. Environ. Microbiol. doi:10.1128/AEM.00354-11 (2011).

Contreras, P.J., et al. Effect of PCR amplicon length on suppressing signals from membrane-compromised cells by propidium monoazide treatment. J. Microbiol. Methods. 87(1), 89-95. (2011).

Elizaquível, P., et al. Quantitative detection of viable foodborne E. coli O157:H7, Listeria monocytogenes and Salmonella in fresh-cut vegetables combining propidium monoazide and real-time PCR. Food Control 25, 704-708. (2012).

Ge, B., et al. Advanced Technologies for Pathogen and Toxin Detection in Foods: Current Applications and Future Directions. J. Assoc. Lab. Automation doi:10.1016/j.jala.2008.12.012 (2009).

Lee, J-L., et al. Comparative Study of the Ability of EMA and PMA to Distinguish Viable From Heat Killed Mixed Bacterial Flora From Fish Fillets. J. Microbiol. Meth. doi: 10.1016/j.mimet.2008.08.008 (2008).

Loozen, G., et al. Live/dead real-time polymerase chain reaction to assess new therapies against dental plaque-related pathologies. Mol. Oral Microbiol. doi: 10.1111/j.2041-1014.2011.00615.x (2011).

Martin, B., et al. Effect of Amplicon Length in Propidium Monoazide Quantitative PCR for the Enumeration of Viable Cells of *Salmonella* in Cooked Ham. Food Anal. Methods. doi: 10.1007/s12161-012-9460-0 (2012).

Nocker, A., et al. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbiol. Meth. 67(2), 310-320 (2006).

Taskin, B., et al. Selective quantification of viable Eschericia coli in biosolids by quantitative PCR with propidium monoazide modification. Appl. Environ. Microbiol. doi:10.1128/AEM.02895-10 (2011).

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.

Related products

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

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