Biotium
Glowing Products for Science™

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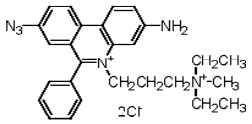
Product Information

PMA™ dye

Catalog Numbers:
40013: PMA dye, 1 mg
40019: PMA dye, 20 mM in dH_2O, 100 uL



Molecular Information:
MW: 511

Color and Form:
40013: orange solid
40019: orange-red liquid

Spectral Properties:
λ_{abs} = 464 nm (before photolysis);
λ_{abs} /λ_{em} = ~510/~610 nm (following photolysis and reaction with DNA/RNA)

Storage and Handling

PMA solid (40013) should be stored at 4 °C or -20 °C protected from light. When stored as recommended, the dye is stable for at least one year from date of receipt. To prepare a 20 mM stock solution, dissolve 1 mg PMA in 98 uL dH_2O. Store the dye solution at -20 °C; when stored as recommended the dye solution is stable for at least six months.

PMA, 20 mM in H_2O (40019) should be stored at -20 °C protected from light. When stored as recommended the dye solution is stable for at least six months from date of receipt. Before each use, briefly centrifuge the vial of PMA to collect the solution at the bottom of the vial to ensure full recovery of product.

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. 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 (Figure 1). 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 useful in the selective detection of viable pathogenic cells by quantitative real-time PCR (Figure 2).

Biotium now offers an improved alternative to PMA called PMAxx™ (40069). PMAxx reduces the PCR signal from dead cell DNA much better than PMA in all bacteria strains we have tested. Thus PMAxx provides the maximum amount of live/dead discrimination by PCR.

When using PMA for viability PCR of gram-negative bacteria, we recommend the use of PMA Enhancer for Gram-Negative Bacteria, 5X Solution (31038). 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.

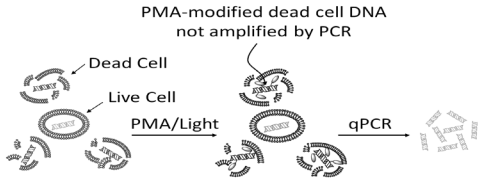


Figure 1. Principle of PMA modification for quantitation of viable bacteria by qPCR. The cell membrane-impermeable PMA dye selectively and covalently modifies DNA from dead bacteria with compromised membranes. Subsequent PCR amplification of PMA-modified DNA templates is inhibited, allowing selective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

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.

Note 1: If you are using the optional PMA Enhancer for Gram Negative Bacteria, please follow the protocol in the product information sheet for that product.

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 or 90°C for 5 min. To confirm killing of bacteria use Biotium's Viability/ Cytotoxicity Assay Kit (30027). Alternatively, 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 plates at 37°C and check for colony growth after 24-48 hours.
5. Pipette 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 **Note 2** below for information on light sources.
9. Pellet cells by centrifuging at 5,000 x g for 10 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 (for primer selection and reaction setup, see **Note 3** and **Note 4**). DNA templates modified with PMA will show delayed amplification by qPCR (Figure 2).

Note 2: For best results, we recommend that the photo-crosslinking be carried out on Biotium's PMA-Lite LED Photolysis Device (see below for product details). 15 min exposure should be sufficient for complete PMA activation.

Commercial halogen lamps (>600 W) for home use have been employed for photo-activating 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, on a rocking platform to ensure continuous mixing. 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 (up to 45° downward slant is OK). Expose samples to light for 5-15 min.

Note 3: 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.

Note 4: 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.

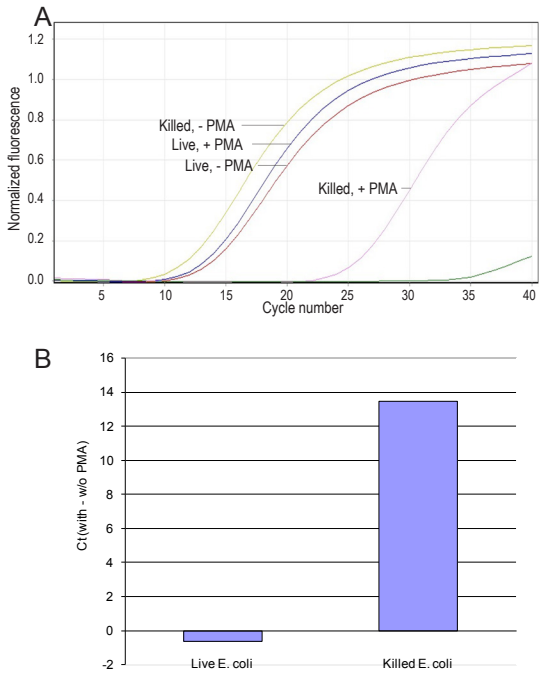


Figure 3. Effect of PMA on qPCR of DNA from live and heat-inactivated *E. coli*. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA-treated live and heat-killed *E. coli*. (B) The ΔCt of live and 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).

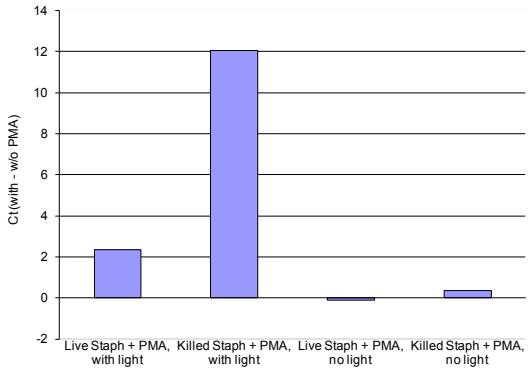


Figure 4. Effect of PMA and light exposure on qPCR of DNA from live and heat-killed *S. epidermidis* (Staph) DNA. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). Samples were exposed to light for 5 minutes after PMA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

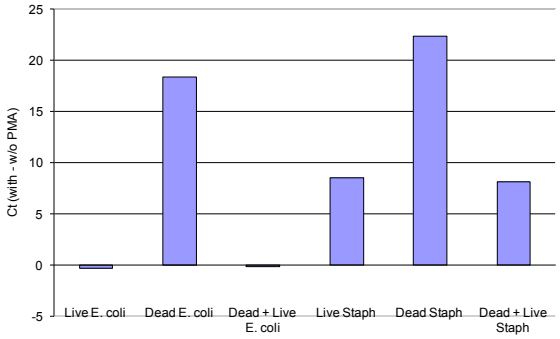


Figure 5. ΔCt of live, heat-killed, and mixed live/killed *E. coli* and *S. epidermis* (Staph) with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding PMA-treated sample (Ct with PMA – Ct without PMA). qPCR was performed using primers against a region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

PMA References

PMA from Biotium has been cited in hundreds of publications. For an updated list of selected references please check the PMA product page on our website.

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).

Light source 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
40069	PMAxx™ Dye, 20 mM in dH2O	100 uL
40015	EMA (ethidium monoazide)	5 mg
E90002	PMA-Lite™ LED Photolysis Device	1 device
31038	PMA Enhancer for Gram-Negative Bacteria, 5X	16 mL
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-PCR bacterial viability kit, E. coli	200 assays
31051	PMA-PCR bacterial viability kit, Listeria	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.