Propidium Mono Azide (PMA)

High affinity photoreactive DNA binding dye

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Propidium Mono Azide (PMA)	High affinity photoreactive DNA binding dye	1 mg	PK-CA707-40013
Propidium Mono Azide (PMA) Solution	PMA Solution, 20 mM in dH_2O	100 µl	PK-CA707-40019

Introduction

PMA is a high affinity photoreactive DNA binding dye that 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 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 pathogenic cells by quantitative real-time PCR (Figure 2).

Molecular Information:

MW: 511

Color and Form:

40013: orange solid

40019: orange-red liquid

Spectral Properties:

λabs = 464 nm (before photolysis);

λabs /λem = ~510/~610 nm

(following photolysis and reaction with DNA/RNA)

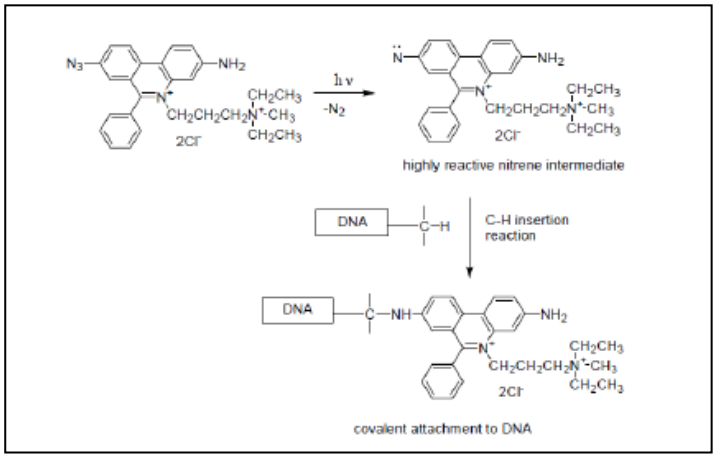

Figure 1. Mechanism of nucleic acid modification by PMA.

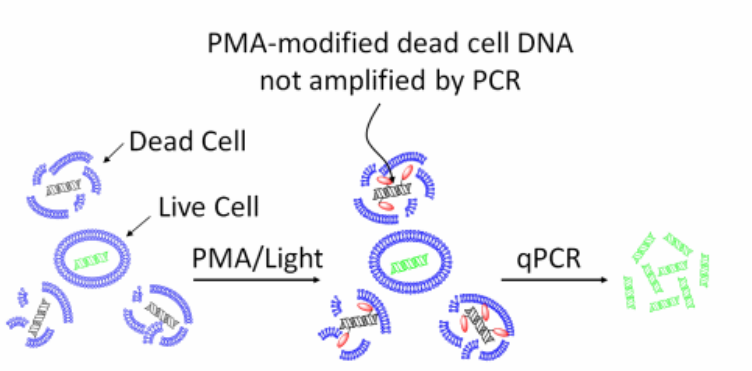

Figure 2. Principle of PMA modification for quantitation of viable bacteria by qPCR.
The cell membrane-impermeable PMA dye selectively and covalently modifies DNA from dead bacteria with compromised membranes. Subsequent PCR amplification of PMA-modified DNA templates is inhibited, allowing selective quantitation of viable bacteria.

Storage and Stability

PMA solid (PK-CA707-40013) 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 µl 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 (PK-CA707-40019) 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. See tips on pipetting the entire 100 µl of dye from the vial below.

Assay Protocol

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_{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 PromoKine's Viability/Cytotoxicity Assay Kit (PK-CA707-30027). Alternatively, plate 10 µl of heat inactivated bacteria on the appropriate media plate, and 10 µl 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 µl aliquots of bacterial culture into clear microcentrifuge tubes.
6. Add the appropriate volume of PMA stock for a final concentration of 50 µM (e.g., 1.25 µl of 20 mM stock in 500 µl).
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 3 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. DNA templates modified with PMA will show delayed amplification by qPCR (Figure 3).

Note 1: 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 2: 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 µl of eluted DNA can be used as a starting point for optimization

Note 3: For best results, we recommend that the photo-crosslinking be carried out on a PMA-Lite LED Photolysis Device (available e.g. at Biotium). 15 minutes 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 minutes.

Procedure for measuring the volume of PMA dye, 20 mM in H_2O (catalog no. PK-CA707-40019)

PMA dye, 20 mM in H_2O is supplied at 100 µl in an amber vial to protect it from light. It can be difficult to remove the entire volume at once to the small volume and the darkness of the amber vial. To measure the entire volume, it is easier to pipette the dye solution in increments.

Note: Due to the light sensitivity of PMA, we recommend dimming the lights in the lab to minimize exposure of PMA to light during this procedure.

1. Warm the vial to room temperature.
2. Place the vial in a microcentrifuge and centrifuge for 30 seconds at ~7000 rpm to collect the solution at the bottom of the vial.
3. Carefully remove the vial from of the centrifuge to keep the solution at the bottom of the vial.
4. Using a 20-200 µl micropipettor, transfer 80 µl of dye into a clean centrifuge tube.
5. Cap the original amber vial and centrifuge for 30 seconds at ~7000 rpm.
6. Using a 1-10 µl micropipettor, transfer 10 µl of the dye to the centrifuge tube from step 4.
7. Cap the original amber vial and centrifuge for 30 seconds at ~7000 rpm.
8. Using a 1-10 µl micropipettor, transfer the remaining dye to the centrifuge tube from step 4.
9. Wrap the centrifuge tube containing the dye in aluminium foil to protect PMA from light during subsequent handling.

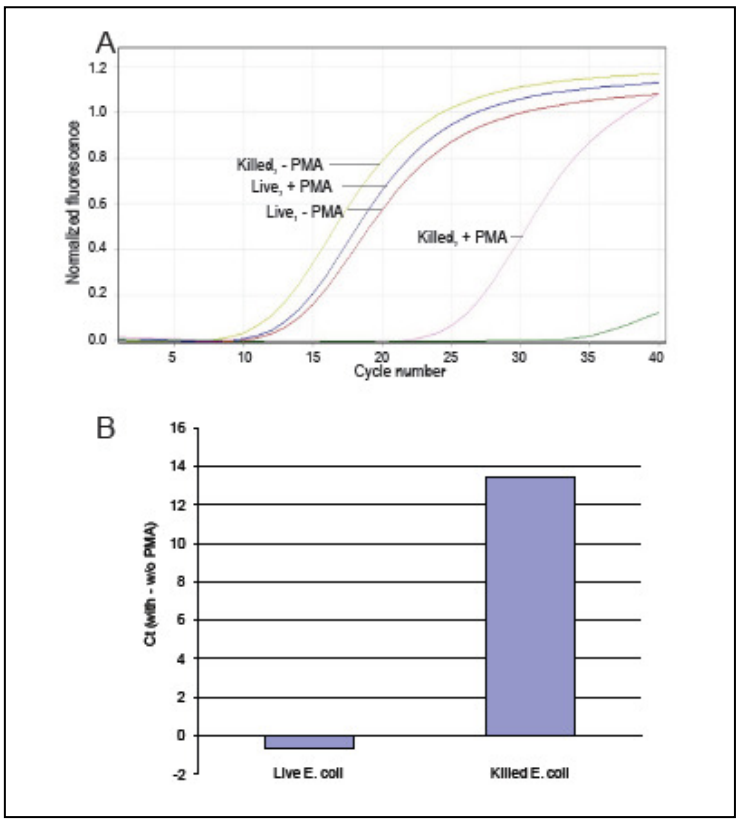

Figure 3. Effect of PMA on qPCR of DNA from live and heat-inactivated *E. coli*. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA-treated live and heat-killed E. coli. (B) The DCt of live and killed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

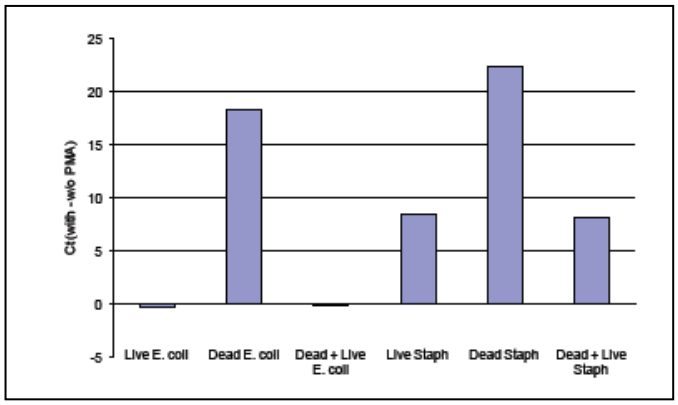

Figure 4 (above). Effect of PMA and light exposure on qPCR of DNA from live and heat-killed *S. epidermidis* (Staph) DNA. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). Samples were exposed to light for 5 minutes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

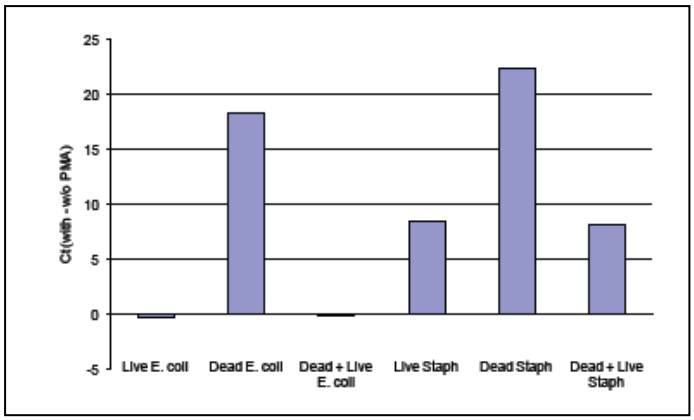

Figure 5. DCt of live, heat-killed, and mixed live/killed *E. coli* and *S. epidermis* (Staph) with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding PMA-treated sample (Ct with PMA – Ct without PMA). qPCR was performed using primers against a region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

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

References

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PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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