

# Product Information

## PMAXx™ dye, 20 mM in dH<sub>2</sub>O

**Catalog Number:** 40069

**Color and Form:** orange-red liquid

### Spectral Properties:

$\lambda_{\text{abs}} = 464 \text{ nm}$  (before photolysis);

$\lambda_{\text{abs}}/\lambda_{\text{em}} = \sim 510\text{--}610 \text{ nm}$  (following photolysis and reaction with DNA/RNA)

### Storage and Handling

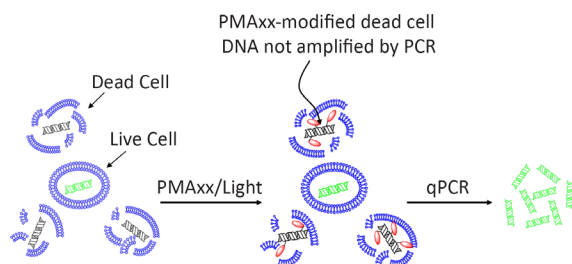
PMAXx, 20 mM in H<sub>2</sub>O should be stored at -20°C protected from light. When stored as recommended the dye solution is stable for at least six months from date of receipt. Before each use, briefly centrifuge the vial of PMAXx to collect the solution at the bottom of the vial to ensure full recovery of product.

### Product Description

PMAXx is a high affinity photoreactive DNA binding dye, developed by Biotium as an improved version of our popular PMA dye. 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). PMAXx 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 microbes by quantitative real-time PCR (Figure 2).

In experiments with laboratory bacterial strains, PMAXx increases the difference between live and dead a further 3-7 Ct compared to PMA. Therefore viability PCR with PMAXx is more effective at discriminating between live and dead bacteria. Because PMAXx works the same way as PMA, it can directly replace PMA in your current PMA-PCR protocol.

When using PMAXx 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 PMAXx, dead cell DNA levels are further decreased, and thus live/dead cell discrimination is improved.



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

### Protocol for treating bacteria with PMAXx for qPCR

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

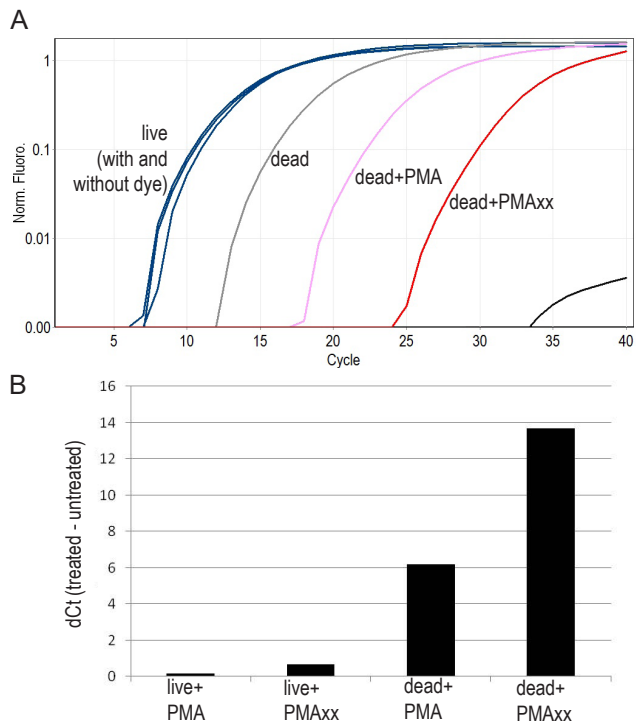
1. Inoculate an appropriate media broth with bacteria (volume is dependent on size of experiment).
2. Shake cultures at 200 RPM at 37°C overnight.
3. Continue culturing bacteria until the OD<sub>600</sub> of the culture is approximately 1.
4. For dead cell control samples, heat inactivate bacteria at 95°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. Prepare a PMAXx working solution (for example, 2.5 mM) by diluting in sterile water, and add the appropriate volume to the samples for a final concentration of 25 uM (e.g., add 5 uL of a 2.5 mM working solution to a 500 uL sample). The PMAXx concentration may need to be optimized depending on strain and sample composition.
7. Incubate tubes in the dark for 5-10 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 PMAXx to DNA. See **Note 1** 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 2** and **Note 3**). DNA templates modified with PMAXx will show delayed amplification by qPCR (Figure 2).

**Note 1:** 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 PMAXx activation.

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, 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 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.

**Note 3:** Part of the proposed mechanism of action of PMAXx is the removal of PMAXx-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 2. Comparison between PMAxx and PMA in viability PCR.** Living and heat-killed *Bacillus subtilis* bacteria were treated with 25  $\mu$ M PMAxx, 25  $\mu$ M PMA, or left untreated. After photolysis and DNA isolation, qPCR was performed using primers against the *Bacillus subtilis* *gyrA* gene. (A) Amplification curves for the real-time PCR reaction performed with DNA from PMAxx- and PMA-treated live and heat-killed *Bacillus subtilis*. (B) The  $\Delta$ Ct of live and killed *Bacillus subtilis* with PMAxx or PMA treatment. The Ct value of untreated sample was subtracted from the corresponding sample treated with PMAxx or PMA ( $dCt = Ct$  with dye – Ct without dye).

### Light source for photoactivation

Biotium offers the PMA-Lite™ LED Photolysis Device for light-induced cross-linking of PMAxx and 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. The PMA-Lite device contains spaces for up to 18 tubes to be illuminated at one time.



### Related products

Cat. No.	Product	Size
40013	PMA dye	1 mg
40019	PMA, 20 mM in dH2O	100 $\mu$ L
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
31053	PMA-PCR bacterial viability kit, Legionella	200 assays
31003-T	Fast EvaGreen® qPCR Master Mix	1 mL
31041-T	Forget-Me-Not™ EvaGreen qPCR Master Mix	1 mL
31043-T	Forget-Me-Not™ Universal Probe Master Mix	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
31062	Yeast Vitality Staining Kit	1000 assays
31063	Yeast Viability Staining Kit	1000 assays
31064	Yeast Live-or-Dye™ Fixable Live/Dead Staining Kit	1000 assays

PMAxx technology is covered by pending patents.

Biotium offers a broad selection of novel fluorescence reagents for molecular and cellular biology. Please visit [www.biotium.com](http://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.