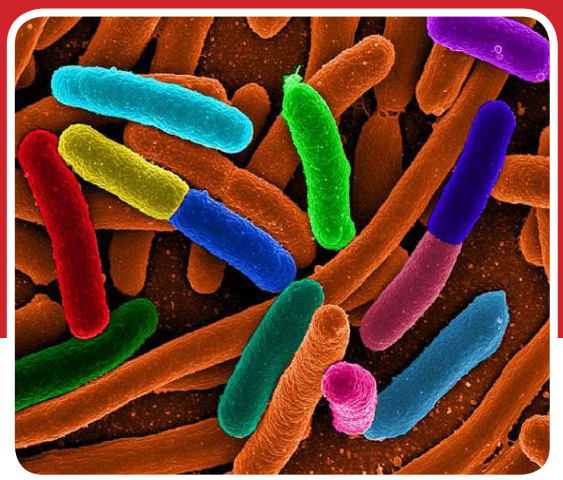

PMAxx™ & PMA

Viability PCR dyes and kits

Live microbial detection by PCR

Viability PCR (v-PCR)

Viability PCR is a powerful technology for the sensitive and rapid detection of viable microorganisms. Unlike time-consuming culturing procedures, qPCR is a fast and sensitive method of detection. However, normal qPCR does not distinguish between live and dead cells. With v-PCR using PMAxx™ or PMA, you get the speed, sensitivity and specificity of PCR, plus quantifiable viability. And because no culturing is required, you can even detect viable but not culturable (VBNC) bacteria.

How does v-PCR work?

PMAxx™ and PMA are photoreactive dyes with high affinity for DNA. The dyes intercalate into dsDNA and form a covalent linkage upon exposure to intense visible light. PMAxx™ and PMA inhibit PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases. Because PMAxx™ and PMA are cell membrane-impermeable, when a sample containing both live and dead bacteria is treated with dye, only dead bacteria with compromised cell membranes are susceptible to DNA modification (Figure 1). In a real-time PCR reaction, dead cell DNA will show delayed amplification and higher Ct than live cells. In a mixed population, v-PCR permits quantitation of cell viability. The v-PCR technology can be applied not only to bacteria but to other cell types as well (see page 2 for details).

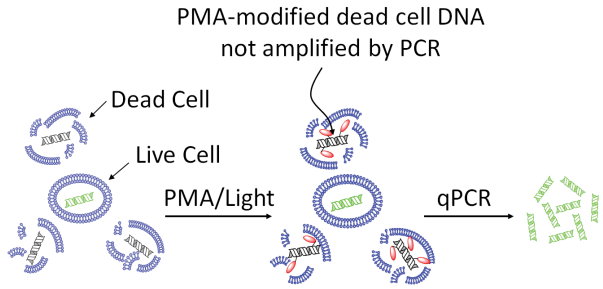

Figure 1. Mechanism of PMA and PMAxx™. The cell membrane-impermeable dyes PMA and PMAxx™ selectively and covalently modify DNA from dead bacteria with compromised membranes while leaving DNA from viable cells intact. Because PMA- and PMAxx™-modified DNA cannot be amplified, subsequent qPCR permits selective quantitation of viable bacteria.

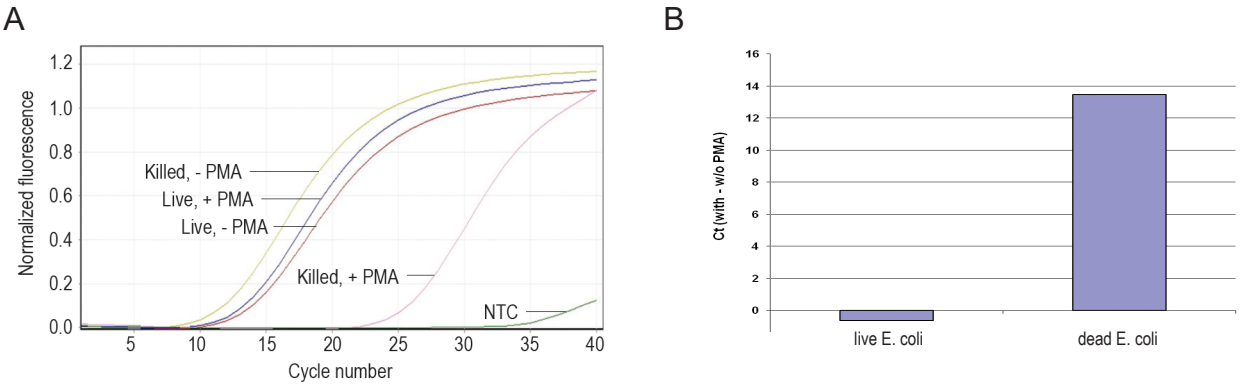

Figure 2. Effect of PMA on qPCR of DNA from live and heat-inactivated E. coli. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA-treated live and heat-killed E. coli. (B) The dCt of live and killed 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).

Biotium

PMAxx™: an improved dye for live/dead discrimination by v-PCR

PMAxx™ vs PMA

Since Biotium developed PMA in 2006, it has been used extensively for many different applications and in hundreds of publications (see box below). PMA has revolutionized the task of bacterial detection by allowing live cell DNA to be specifically quantified. However there are cell types and conditions in which dead cell DNA inactivation by PMA is incomplete, which could lead to false positive results. After extensive testing, the scientists at Biotium have invented a new dye called PMAxx™ that has the same spectral properties and is even more effective than PMA at live/dead discrimination by viability PCR (Figure 3).

For experienced users of PMA, PMAxx™ can be used in your current PMA-PCR protocol. PMAxx™ is also compatible with our PMA-Lite™ (see back page) and PMA Enhancer for Gram-Negative Bacteria (see next page).

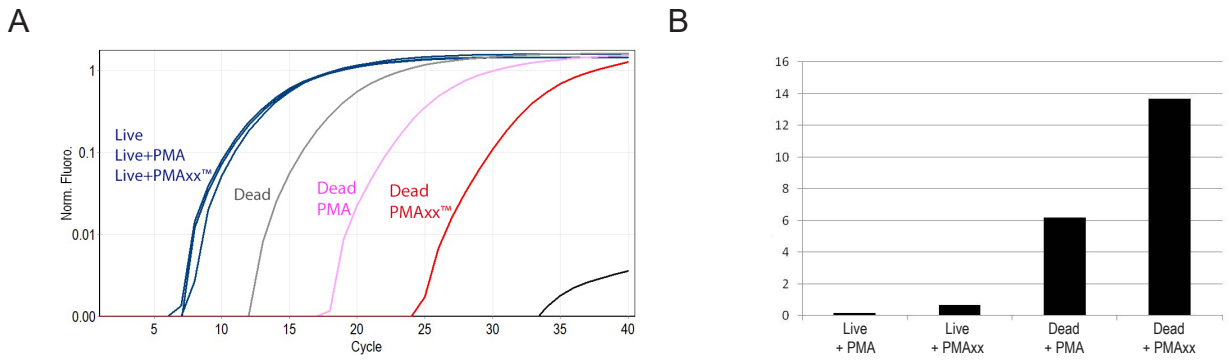

Figure 3. A) Live or heat-killed Bacillus subtilis were treated with 25 uM PMA or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 500-bp fragment of B. subtilis DNA. Treatment of the cells with viability dye did not affect the amplification of DNA from live cells, but caused a decrease in Ct in dead cells. qPCR of dead cells treated with PMAxx™ showed a significant further delay (>7 Ct) compared to dead cells treated with PMA. B) From the qPCR data in panel A), dCt values were calculated by subtracting the Ct without dye from the Ct with dye. While both PMA and PMAxx™ specifically reduce the amplifiable DNA from dead cells, PMAxx™ improved live/dead discrimination in all bacteria tested.

v-PCR with PMA has been extensively validated

PMA from Biotium has been cited in hundreds of publications, and in dozens of different organisms and applications, such as:

- Dozens of different bacteria species, including Legionella, Listeria, Mycobacteria, Bacillus, Staphylococcus, Vibrio, Enterococcus, Salmonella, Helicobacter, Bacteroides, Pseudomonas, Chlamydia, E. coli, and many others
- Fungi and yeast, including Candida albicans, Saccharomyces cerevisiae, Zygosaccharomyces, Aspergillus, and other fungi
- Several viruses, including Hepatitis A, Rotavirus, Norovirus, bacteriophage, and enteroviruses
- Population studies using deep sequencing
- Food safety testing of meat, dairy and produce
- Water quality testing of oceans, lakes, water tanks and wastewater
- Microbes in space stations
- Medical sample testing
- Removal of contaminating extracellular DNA from samples before PCR

Strain-Specific Bacterial Viability PCR Kits

All-In-One Kits

Cells can be treated with PMA or PMAxx™ prior to any quantitative PCR reaction, which is ideal for users that already have their an established PCR assay for their strain of interest. However for maximal convenience, we also offer bacterial strain-specific viability PCR kits for several popular bacterial strains. These kits are designed for the selective detection of viable bacteria from a specific strain using a viability dye (PMA or PMAxx™) and real-time PCR. The kits contain either PMAxx™ or PMA viability dye, our exceptionally sensitive Forget-Me-Not™ qPCR Master Mix, and a set of validated PCR primers for detection of selected strains of bacteria that are of widespread interest to food safety, public health, and/or antibacterial research.

Kits include:

- Your choice of PMAxx™ or PMA viability dye
- Forget-Me-Not™ qPCR Master Mix
- ROX reference dye
- Validated strain-specific primer set
- 5X PMA Enhancer for Gram-Negative Bacteria (gram-negative strains only)

Kits available for:

- Salmonella enterica
- Escherichia coli
- Escherichia coli O157:H7
- Listeria monocytogenes
- Legionella pneumophila
- Mycobacterium tuberculosis
- Staphylococcus aureus
- Methicilin resistant Staphylococcus aureus (MRSA)

Example data from the Salmonella Viability PCR Kit

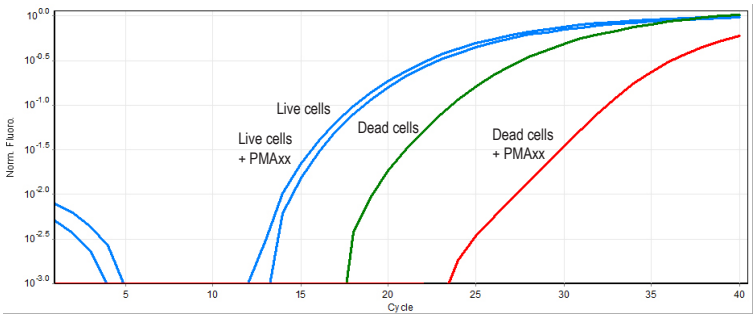

Figure 4. A culture of Salmonella enterica was split and half of the cells were heat-killed at 95°C. Live and dead bacteria were then either left untreated or treated with 10 uM PMAxx™ viability dye, followed by light treatment with the PMA-Lite. DNA was purified from each sample and used as the template in a qPCR reaction using the Salmonella-specific InvA primers that are provided in the Salmonella Viability PCR Kit. Treatment with PMAxx™ caused a drastic reduction in the amplification of dead cell DNA but had no effect on live cell DNA.

Enhancer improves v-PCR of mildly heat killed E. coli

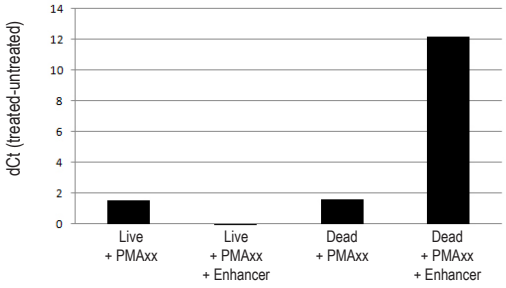

Figure 5. E. coli were killed with mild heat treatment (56°C for 3 hrs) and treated with PMAxx™ or PMAxx™+Enhancer, followed by light exposure using PMA-Lite™, DNA purification, and qPCR with Fast EvaGreen® qPCR Master Mix. dCt values were calculated by subtracting the Ct without dye from the Ct with dye. Only dead cells treated with PMAxx™+Enhancer showed a large dCt, indicating that the dye successfully inhibited PCR of dead cell DNA.

PMA Enhancer for Gram-Negative Bacteria

Under some conditions such as mild heat treatment, bacteria may be dead but retain intact membranes that have lower permeability to many viability dyes. Biotium has developed an Enhancer for use with gram-negative bacteria that can greatly improve live/dead discrimination with PMAxx™ or PMA (Figure 5).

Benefits of Enhancer include:

- Improved live/dead discrimination of gram-negative bacteria.
- Drastic improvement in PMAxx™ or PMA efficiency in cases of mildly-killed bacteria.

LED Photolysis Devices

PMA-Lite™

- Holds up to 18 microcentrifuge tubes
- Bright, long-lasting LEDs with 465-475 nm emission
- Designed for efficient photoactivation of PMA, PMAxx™ and similar dyes
- Internal fan to ensure temperature below 37°C
- Four timer settings

Glo-Plate™ Blue

- Flat illumination surface fits microplates
- Bright, long-lasting LEDs with 465-475 nm emission
- Designed for efficient photoactivation of PMA, PMAxx™ and similar dyes
- Surface stays cool during light exposure
- Four timer settings

Ordering Information

Cat. #	Product name	Unit size
40069	PMAxx™ dye, 20 mM in dH_2O	100 uL
40013	PMA dye	1 mg
40019	PMA dye, 20 mM in dH_2O	100 uL
E90002	PMA-Lite™ LED Photolysis Device	1 device
E90004	Glo-Plate™ Blue	1 device
31038	PMA Enhancer for Gram-Negative Bacteria	16 mL
31033	Real-Time Bacterial Viability Kit-Salmonella (InvA)	200 assays
31034	Real-Time Bacterial Viability Kit-M. tuberculosis (groEL2)	200 assays
31035	Real-Time Bacterial Viability Kit-Staph. aureus (nuc)	200 assays
31036	Real-Time Bacterial Viability Kit-MRSA (mecA)	200 assays
31050	Real-Time Bacterial Viability Kit-E. coli (uidA)	200 assays
31037	Real-Time Bacterial Viability Kit-E. coli O157:H7 (Z3276)	200 assays
31051	Real-Time Bacterial Viability Kit-Listeria monocytogenes (hly)	200 assays
31053	Real-Time Bacterial Viability Kit-Legionella pneumophila (mip)	200 assays

Related products

Cat. #	Product name	Unit size
40015	Ethidium monoazide, bromide (EMA)	5 mg
31042-T	Forget-Me-Not™ qPCR Master Mix	100 reactions
31044-T	Forget-Me-Not™ Universal Probe Master Mix	100 reactions
32001	Bacterial Viability and Gram Stain Kit	200 assays
32000	Live Bacterial Gram Stain Kit	800 assays
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells	100-1000 assays
32002-T -32009-T	Live-or-Dye™ Fixable Viability Staining Kits	50 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

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