PMA™ and PMAxx™ Dyes and Ready-To-Use Kits

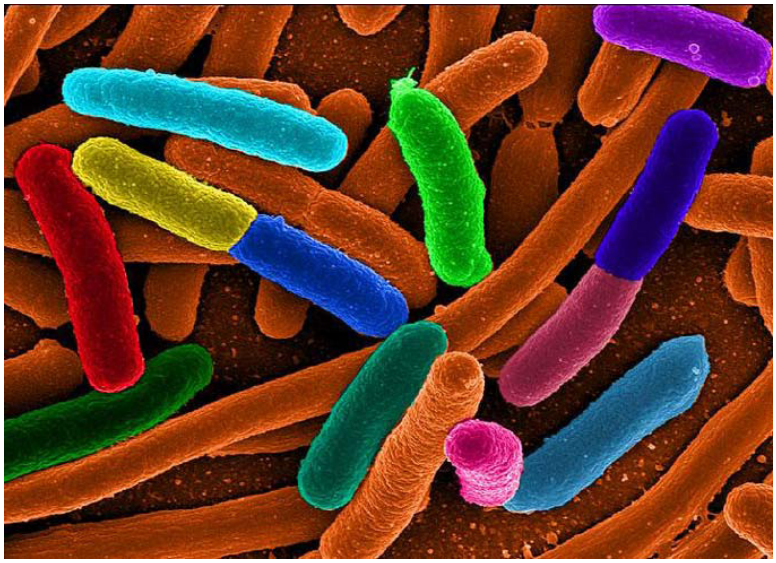
Live Bacterial Detection by viability PCR- *Fast and Specific*

Viability PCR using PMA™ or PMAxx™ has many uses including:

- Food safety
- Probiotic detection
- Environmental testing
- Infectious disease testing
- Microbiology research

Food safety

Environmental testing



Microbiology research

Biotium
Glowing Products for Science™
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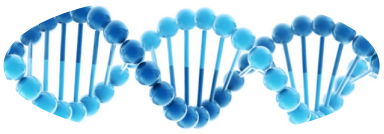
Introduction to PMA™ and PMAxx™



The ability to distinguish viable from dead bacteria is vital for many applications, including food safety, environmental testing, and infectious disease research. Traditional detection methods based on bacterial culturing are time-consuming and insensitive. Detection based on PCR is a more rapid, specific, and sensitive method. However, PCR alone cannot distinguish live from dead cells. The novel DNA-modifying dye propidium monoazide (PMA™) developed by Biotium overcame this problem, and now the scientists at Biotium have developed an even more selective dye, PMAxx™.

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 modified template amplification by DNA polymerases (see Figure 1).

Pre-treating a bacterial sample with PMAxx™ or PMA™ prior to PCR analysis permits one to selectively detect viable bacteria in a highly sensitive and reliable manner. Because the dyes are cell membrane-impermeable, when a sample containing both live and dead bacteria is treated with PMAxx™ or PMA™, only dead bacteria with compromised cell membranes are susceptible to DNA modification. In a real-time PCR reaction, dead cell DNA will show delayed amplification and higher Ct than live cells (Figure 2). In a mixed population, qPCR permits quantitation of cell viability. The PMA™-qPCR technology can be applied not only to bacteria but to other cell types as well.

Did You Know?

PMA™ from Biotium has been cited in hundreds of publications.

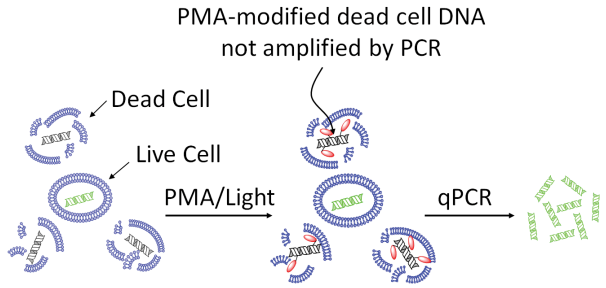


Figure 1. Mechanism of PMA™ and PMAxx™. The cell membrane-impermeable dyes PMA™ and PMAxx™ selectively and covalently modify DNA from dead bacteria with compromised membranes while leaving DNA from viable cells intact. Because PMA™- and PMAxx™-modified DNA can not be amplified, subsequent qPCR permits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

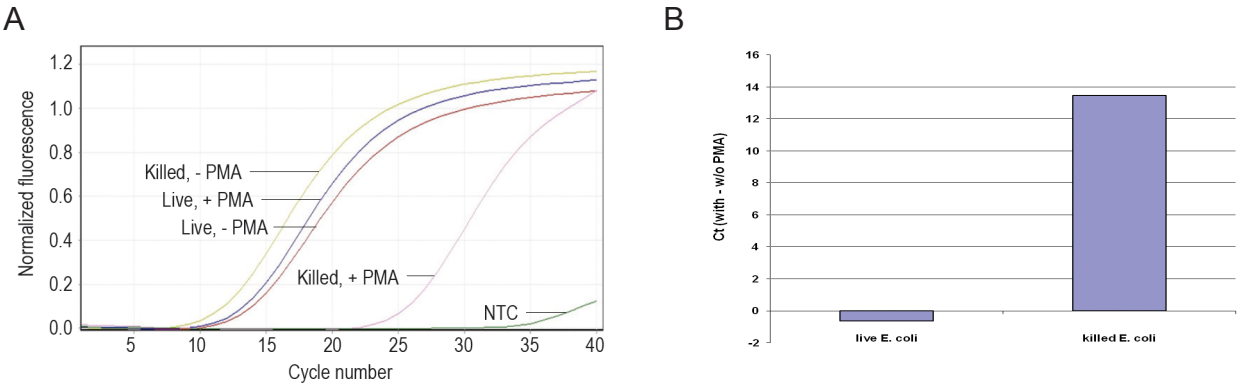


Figure 2. Effect of PMA™ on qPCR of DNA from live and heat-inactivated E. coli. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA™-treated live and heat-killed E. coli. (B) The dCt of live and killed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

PMAxx™

A new and improved viability PCR dye from the inventors of PMA™

Since Biotium developed PMA™ in 2006, it has been used extensively for many different applications and in hundreds of publications. 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. In fact, because of its high activity, you may be able to use PMAxx™ at a lower concentration. PMAxx™ is also compatible with our PMA-Lite™ (see below) and PMA™ Enhancer for Gram-Negative Bacteria (see next page).

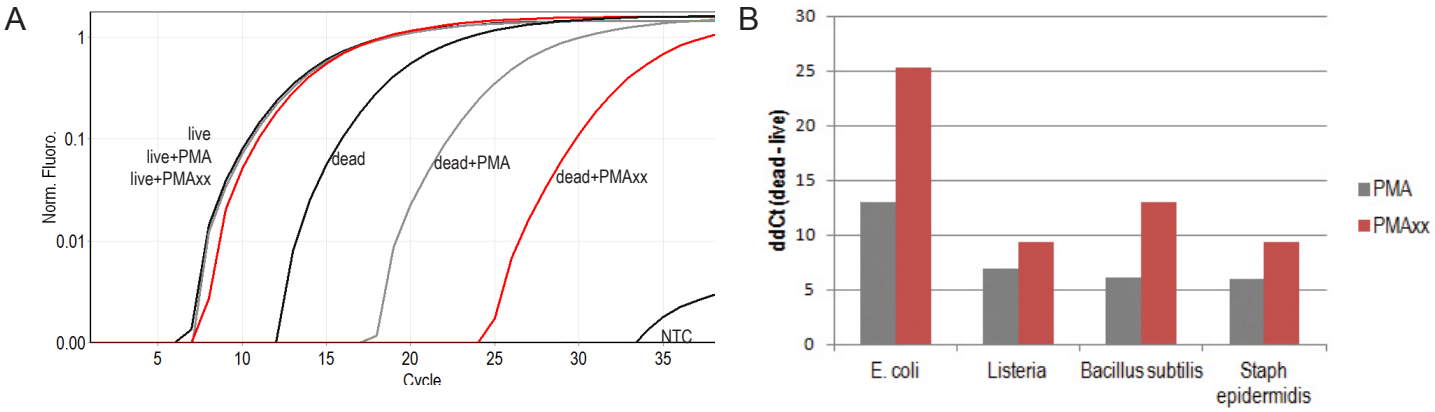


Figure 3. PMAxx™ allows for better discrimination of live and dead bacteria in viability PCR. (A) Live or heat-killed Bacillus subtilis were treated with 25 uM PMA™ or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 500-bp fragment of B. subtilis DNA. Dead cells treated with PMAxx™ showed a significant further delay (>7 Ct) compared to dead cells treated with PMA™. (B) Each of the indicated bacteria were treated with 25 uM PMA™ or PMAxx™, as described in (A). dCt values were calculated by subtracting the Ct without dye from the Ct with dye. ddCt values were calculated by subtracting the dCt for live cells from the dCt for dead cells. PMAxx™ improved live/dead discrimination in all bacteria tested, with improvements in ddCt ranging from 3 to 12.

PMA-Lite™ LED Photolysis Device

PMA-Lite™ is specifically designed for photoactivation of PMA™ and other similar dyes. Receive a free vial of PMA™ dye when you purchase a PMA-Lite™ LED Photolysis Device.

Features:

- Provides even illumination to up to 18 microcentrifuge tubes
- Internal fan to ensure a temperature of <37°C.
- Four timer settings for 10, 15, 20 or 30 minutes of illumination.
- Long-lasting LEDs with 465-475 nm emission for efficient activation of PMA™, PMAxx™, EMA or other similar azido dyes.

PMA™ Real-Time PCR Bacterial Viability Kits

PMA™-PCR kits are designed for selective detection of viable bacteria from a specific strain using PMA™ dye and real-time PCR. The kits contain PMA™ dye, Fast EvaGreen® qPCR Master Mix, and 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:

- PMA™ dye
- Fast EvaGreen® qPCR Master Mix
- ROX reference dye
- Validated PCR primers for specific bacterial strain
- 5X PMA™ Enhancer for Gram-Negative Bacteria (gram-negative strains only)

Kits available for:

- Salmonella enterica
- Staphylococcus aureus
- MRSA
- Escherichia coli & Escherichia coli O157:H7
- Mycobacterium tuberculosis
- Listeria monocytogenes

Don't see your favorite bacteria? Let us know at techsupport@biotium.com

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 PMA™ than those of boiled bacteria, for example. This could result in an overestimate of live bacteria. Biotium has developed an Enhancer for use with gram-negative bacteria that can greatly improve live/dead discrimination with PMA™ or PMAxx™.

Benefits of Enhancer include:

- Improved live/dead discrimination of gram-negative bacteria.
- Drastic improvement in PMA™ efficiency in cases of mildly-killed bacteria.
- Allows the use of PMA™ at a lower concentration, thus potentially decreasing toxicity and saving money.

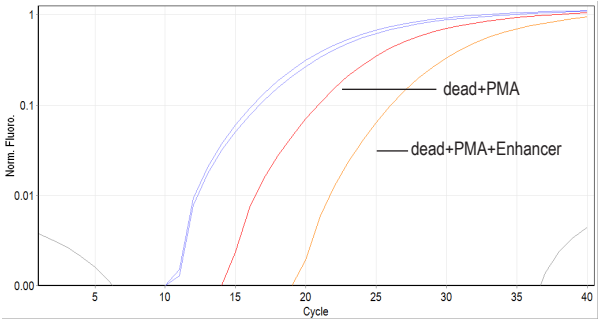


Figure 4. Enhancer improves live/dead discrimination by PMA™ in viability PCR. Mildly heat-treated E. coli were treated with PMA™ or PMA™+Enhancer, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 377-bp fragment of E. coli DNA. Dead cells treated with PMA™+Enhancer showed a significant further delay in Ct compared to dead cells treated with PMA™ alone.

Ordering information

Cat. #	PMA™-related products	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
31038	PMA™ Enhancer for Gram-Negative Bacteria	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
31050	PMA™-PCR Bacterial Viability Kit-E. coli	200 assays
31037	PMA™-PCR Bacterial Viability Kit-E. coli O157:H7	200 assays
31051	PMA™-PCR Bacterial Viability Kit-Listeria monocytogenes	200 assays

Cat. #	Other products	Unit Size
40015	Ethidium monoazide, bromide (EMA)	5 mg
31003-T	Fast EvaGreen® qPCR Master Mix	100 reactions
31041-T	Forget-Me-Not™ qPCR Master Mix	100 reactions
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells	100-1000 assays
32001	Bacterial Viability and Gram Stain Kit	200 assays

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF™dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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