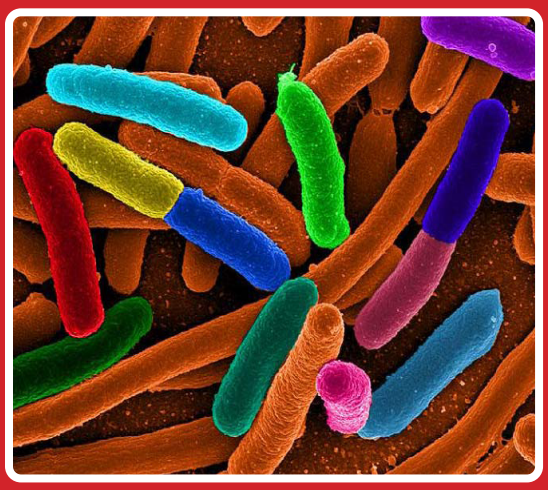


Viability PCR

A powerful PCR-based method for analyzing microbial viability.

DOMINIQUE DUTSCHER SAS

Viability PCR (v-PCR)

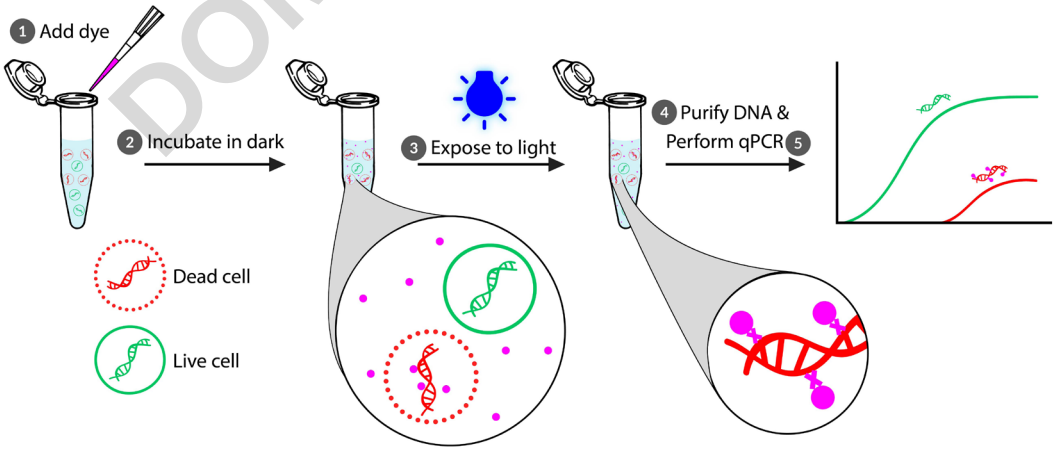
Viability PCR is a powerful technology for the sensitive and rapid detection of viable microorganisms. Unlike time-consuming culturing procedures, qPCR is a fast and sensitive method of detection. However, normal qPCR does not distinguish between live and dead cells. With v-PCR using PMAxx™ or PMA, you 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, or bacteria in mixed populations or complex samples.

How does v-PCR work?

PMAxx™ and PMA are photoreactive dyes that bind to DNA. The dyes intercalate into DNA 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 impermeant, 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 to bacterial as well as other cell types including fungi, yeast, and viruses.

v-PCR has been extensively validated for:

- 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 shotgun metagenomics sequencing using most major NGS platforms
- Food safety testing of meat, dairy and produce
- Water quality testing of oceans, lakes, water tanks and wastewater



Download list of bacterial strains validated for viability PCR

Figure 1. Overview of v-PCR method for sensitive, specific, and rapid detection of viable microbes.

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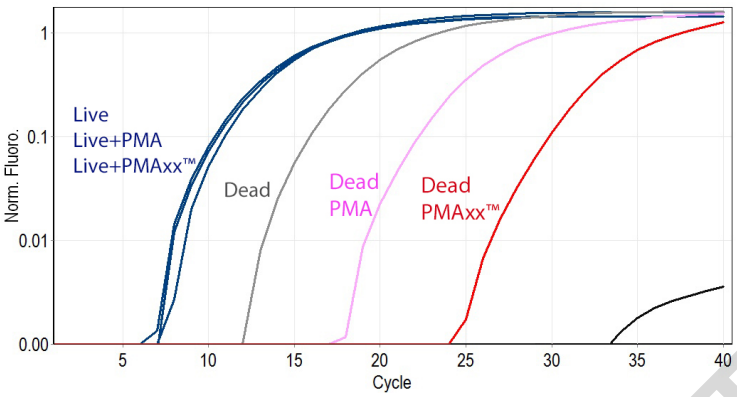
PMAxx™ Next-Generation v-PCR Dye

PMAxx™ vs 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 accurately 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 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 2).

For experienced users of PMA, PMAxx™ can be used in your current PMA-PCR protocol. PMAxx™ is also compatible with our PMA-Lite™ 2.0 LED Photolysis Device (see back page) and PMA Enhancer for Gram-Negative Bacteria.

A



B

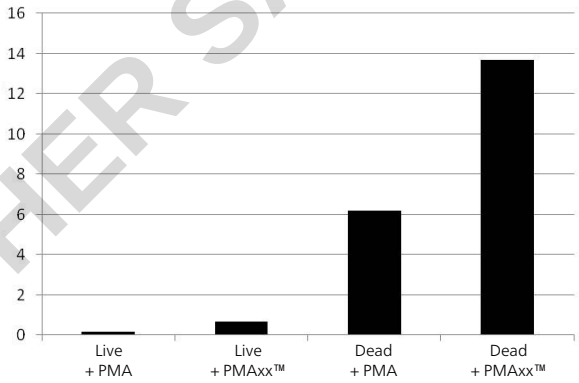


Figure 2. A) Live or heat-killed *Bacillus subtilis* were treated with PMA or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Fast EvaGreen® qPCR Master Mix was used to amplify a 500-bp fragment of *B. subtilis* DNA. B) dCT values from qPCR data in panel A. While both PMA and PMAxx™ specifically reduce the amplifiable DNA from dead cells, PMAxx™ dramatically improved live/dead discrimination by delaying Ct in dead bacteria by a further 7 cycles compared to PMA.

Viability PCR Starter Kits

The customizable Viability PCR Starter Kits are the easiest way to get started in v-PCR. These starter kits contain the materials needed for selective detection of viable cells using either PMA or PMAxx™ viability dye and qPCR. These kits can be used with any cell type of your choosing. All of the kits contain Forget-Me-Not™ EvaGreen® qPCR Master Mix, and your choice of PMA or PMAxx™ dye. You also have the choice of selecting a kit with or without the Enhancer for Gram Negative Bacteria (not to be used with other cell types). The user will need to supply their own primers to amplify their species of interest.

Kits include:

- Your choice of PMAxx™ or PMA viability dye
- Forget-Me-Not™ EvaGreen® qPCR Master Mix
- ROX reference dye
- PMA Enhancer for Gram-Negative Bacteria (for use with gram-negative strains only)

Not included but required:

- Primers to amplify DNA from your cell type of interest
- DNA extraction kit or reagents

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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 an established PCR assay for their strain of interest. However for added 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 EvaGreen® Dye-based 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 available for:

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

Kits include:

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

Example data from the Salmonella Viability PCR Kit

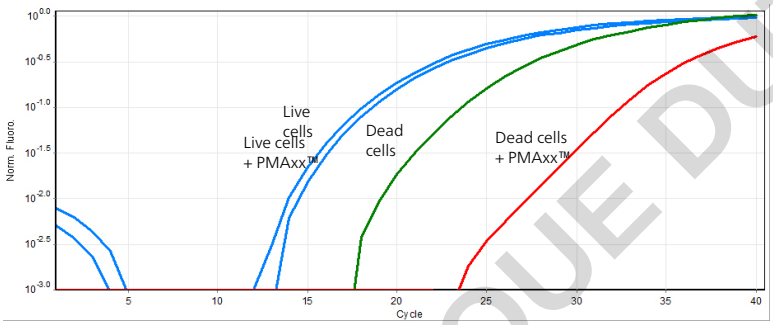


Figure 3. v-PCR of *Salmonella enterica* using the Real-Time Bacterial Viability Kit-*Salmonella* (InvA) with PMAxx™ and photoactivation with the PMA-Lite™. PMAxx™ caused a drastic reduction in the amplification of dead cell DNA with minimal effect on live cell DNA amplification.

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

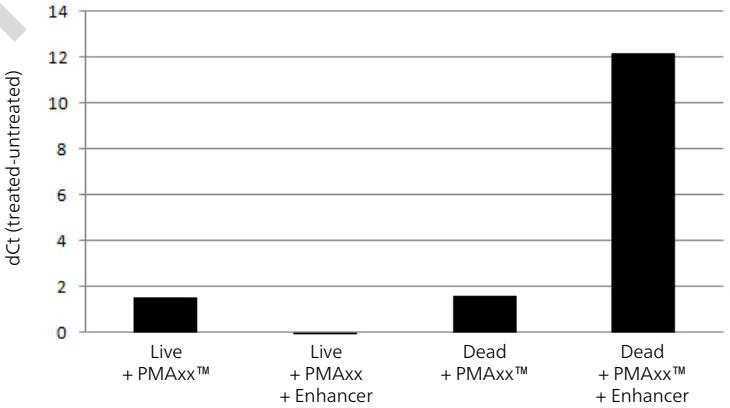


Figure 4. *E. coli* were killed with mild heat treatment and treated with PMAxx™ with or without PMA Enhancer, followed by photoactivation with PMA-Lite™ and qPCR with Fast EvaGreen® qPCR Master Mix. Only dead cells treated with PMAxx™ + Enhancer showed a large delay in Ct, indicating that the dye successfully inhibited PCR of dead cell DNA only when Enhancer was used.

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 solution for use with gram-negative bacteria that can greatly improve live/dead discrimination with PMAxx™ or PMA (Figure 4).

Benefits of Enhancer include:

- Improved live/dead discrimination of gram-negative bacteria.
- Drastic improvement in PMAxx™ or PMA efficiency with treatments that are less disruptive to bacterial membranes.

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LED Photolysis Device

PMA-Lite 2.0™ LED Photolysis Device

- Bright and uniform illumination for up to 18 tubes
- Long-lasting LEDs with ~470 nm emission
- Designed for efficient photoactivation of PMA, PMAxx™, EMA, and similar dyes
- Internal fan to ensure temperature stays below 37°C
- Timer for 5-45 minutes of photoactivation



Related Products

Cat. No.	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
40107-40113	BactoView™ Dead Stains	20 uL or 100 uL of 500X
32019-32020	BactoView™ Viability Kits	1 kit
40119-40120	BactoSpore™ Bacterial Stains	20 uL or 100 uL of 500X
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

Ordering Information

Cat. No.	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
E90006	PMA-Lite™ 2.0 LED Photolysis Device	1 device
31038	PMA Enhancer for Gram-Negative Bacteria	16 mL
31075, 31076	Viability PCR Starter Kits	200 assays
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

EvaGreen is a registered trademark of Biotium, Inc. Forget-Me-Not, PMAxx, PMA-Lite, Live-or-Dye are trademarks of Biotium, Inc. PMAxx is covered by granted and/or pending US and international patents.

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