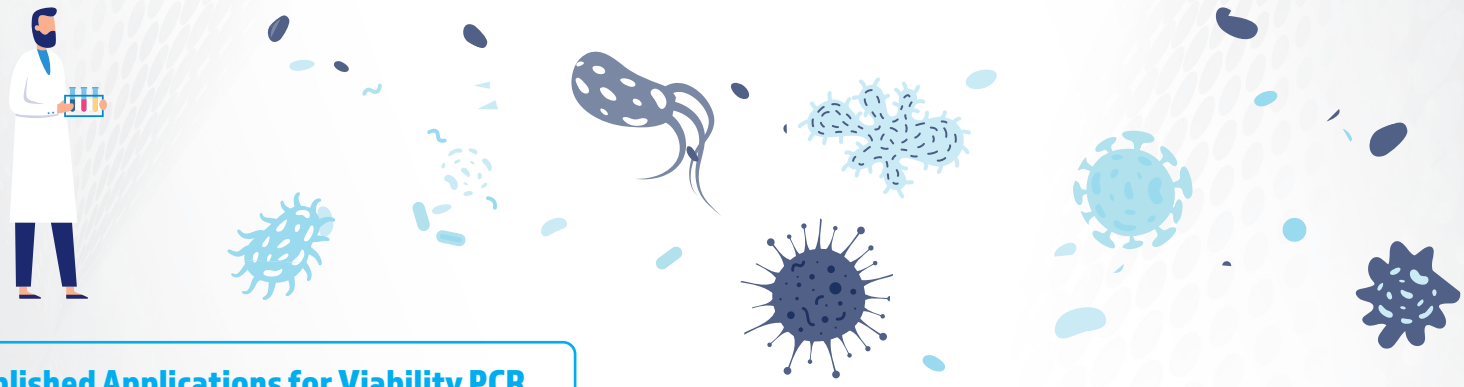
Viability PCR: A Powerful PCR-Based Method for Rapid & Sensitive Analysis of Microbial Viability

Show Me the Microbes

Reliable detection of living microbes and viruses is of widespread interest across numerous sectors including basic research, food safety, agriculture, and public health. Early methods to detect viable microorganisms were based on culturing and microscopic observation, which is time-consuming, non-specific, and labor intensive. In addition, certain organisms cannot be cultured with current technology. Consequently, these viable but non-culturable (VBNC) species require molecular measures of viability. A rapid and reliable culture-free method for analyzing microbial viability didn't arrive until the advent of viability PCR (v-PCR). Since 2006, Biotium has pioneered the field of v-PCR with the development of PMA (Propidium Monoazide), a photoreactive, membrane-impermeant, DNA binding dye with superior dead cell selectivity over traditional culture-based methods and the previous EMA (Ethidium Monoazide) v-PCR dye.

Advantages of Viability PCR with PMA & PMAxx™

- Sensitive and rapid viability analysis without time-consuming culturing, microscopy, or colony counting
- Allows strain-specific viability analysis
- Validated and published for dozens of bacteria, fungi and viral species
- Compatible with Next-Generation Sequencing applications
- Suitable for complex sample types including soil, feces, water/wastewater, biological specimens, and food



Published Applications for Viability PCR

- Microbiome Studies
- Environmental Testing
- Probiotic Research
- Clinical Testing
- Food & Water Sanitation
- Wine Fermentation
- Microbial Sequencing

View full list of PMA and PMAxx™ publications.

An Industry Leader in v-PCR

PMA is the v-PCR dye of choice among industry leaders, and has been validated in hundreds of peer-reviewed publications and numerous strains of bacteria, fungi, and viruses. Early studies focused on v-PCR in bacteria, validating the procedure in dozens of different bacterial strains such as *E. coli*, *Listeria*, *Legionella*, *Lactobacillus*, *M. tuberculosis*, *Chlamydia*, *H. pylori*, and many other strains with relevance to human health. v-PCR has been most widely used in bacteria, but there are also dozens of publications using v-PCR in yeast and fungi, viruses, as well as a variety of other cell types such as archaea, amoeba, and parasites.

How Does v-PCR Work?

v-PCR intuitively merges the specificity and sensitivity of qPCR-based methods with a dead cell selective dye. The technique is extremely versatile and can be applied to numerous species of bacteria, eukaryotes, viruses, and archaea. The basic workflow is shown in Figure 1 and may be adapted to many different sample types and experimental set-ups. The process begins by first treating a cell sample with PMA dye. Because PMA is membrane impermeant, the dye only enters dead cells with damaged membranes, where it binds to DNA with high affinity. After dye incubation, the sample is then exposed to intense light. This photolysis step converts the dye to a highly reactive nitrene intermediate that covalently modifies DNA hydrocarbons.

The mechanism that underlies the distinction of dead microbes from live ones is two-fold. The DNA that is crosslinked to dye is less soluble and precipitates during DNA isolation, resulting in a lower recovery of modified DNA. In addition, the covalent modification on any remaining DNA inhibits downstream PCR amplification, resulting in delayed Ct values in qPCR compared to unmodified DNA. The technology also can be applied to downstream sequencing applications for population analysis.

The advantage of v-PCR is that it allows specific and quantitative strain detection without the need to cultivate the target organisms. For this reason, v-PCR can be used for viable but non-culturable, or VBNC bacteria. There are also many publications that have adapted the PMA protocol to analyze large dilute samples or complex samples such as soil or feces.

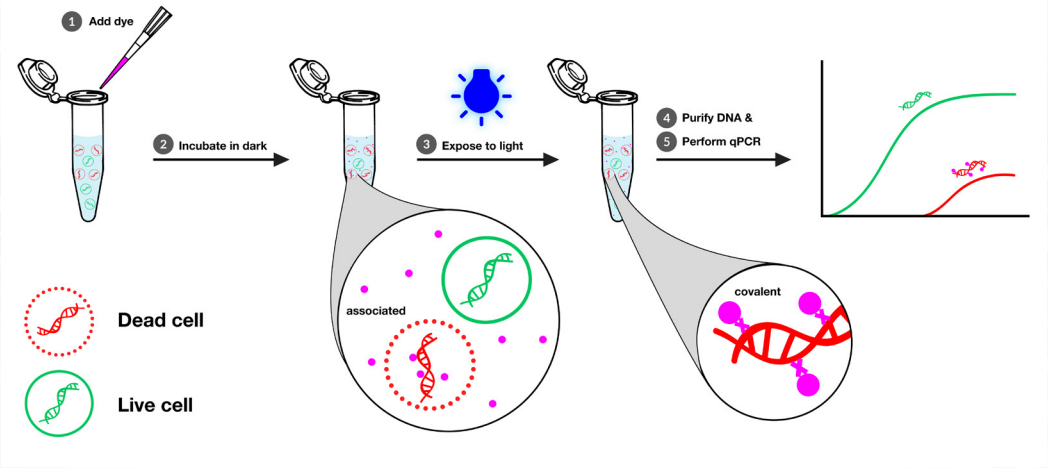


Figure 1. Overview of PMA and PMAxx™ v-PCR dyes for sensitive, specific, and rapid detection of viable microbes.

PMAxx™, a New and Improved v-PCR Dye

Biotium has further advanced the field of v-PCR with the release of PMAxx™, a superior next-generation v-PCR dye that provides greater live/dead discrimination. PMAxx™ more effectively suppresses DNA amplification from dead cells, demonstrating an increase of dead cell selectivity by 3-7 Ct in qPCR compared to PMA in laboratory bacterial strains (Figure 2).

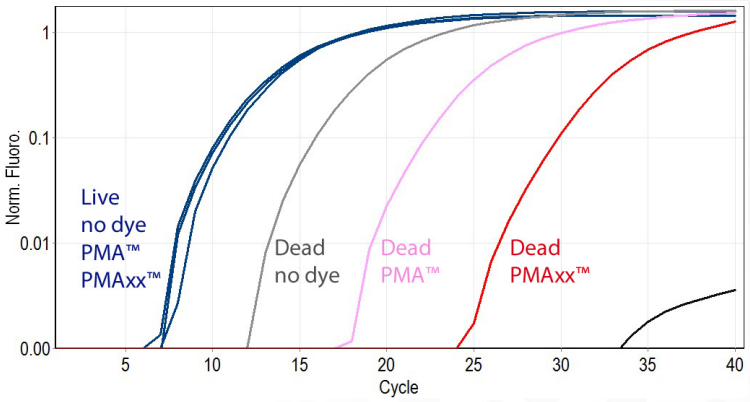


Figure 2. A) Live or heat-killed *Bacillus subtilis* were treated with 25 uM PMA or PMAxx™, followed by exposure with the PMA-Lite™ and DNA purification. Treatment of the cells with viability dye did not affect the amplification of DNA from live cells, but caused a decrease in Ct in dead cells. qPCR of dead cells treated with PMAxx™ showed a significant further delay (>7 Ct) compared to dead cells treated with PMA.

Improve Dead Cell Selectivity for Gram-Negative Bacteria with the PMA Enhancer Solution.

Under some conditions such as mild heat treatment, bacteria may be dead but retain relatively intact membranes with low permeability to viability dyes like PMA. Biotium has developed PMA Enhancer solution for use with gram-negative bacteria to greatly improve live/dead discrimination. When added to the sample before the addition of PMA or PMAxx™, the enhancer solution can dramatically reduce amplification from dead cells without affecting the signal from live cells (Figure 3).

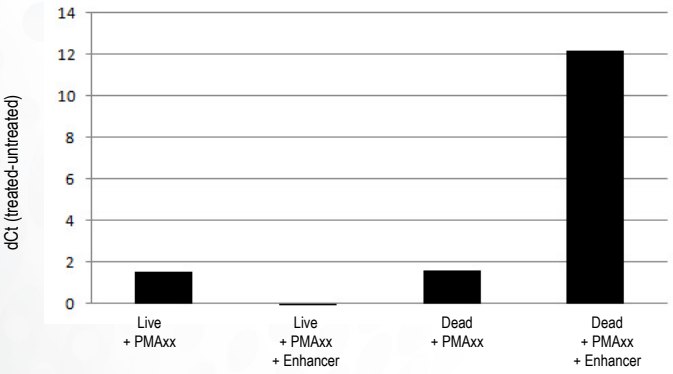


Figure 3. *E. coli* were killed with mild heat treatment (56°C for 3 hrs) and treated with PMAxx™ or PMAxx™ + Enhancer, followed by light exposure using PMA-Lite™, DNA purification, and qPCR amplification. dCt values were calculated by subtracting the Ct without dye from the Ct with dye. Only dead cells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

PMA-Lite™ Device for Convenient and Precise Photoactivation

The PMA-Lite™ LED Photolysis Device allows for uniform and controlled light treatment of your PMA or PMAxx™ treated samples (Figure 4). The PMA-Lite™ is suited for up to 18 microcentrifuge tubes and includes an internal fan to maintain temperatures below 37°C.

Figure 4. PMA-Lite™ LED Photolysis Device.

Starter Kits and Strain Specific v-PCR kits

New to v-PCR? Biotium offers Viability PCR Starter Kits complete with PMA or PMAxx™, exceptionally sensitive EvaGreen® qPCR master mix, and ROX reference dye. Kit variations containing the PMA Enhancer Buffer are also available for testing gram-negative bacteria. Each kit can be used for a variety of cell types and contains enough reagents to treat 80 bacterial cultures and perform 200 PCR reactions. If you are looking at a popular bacterial strain, Biotium also offers strain-specific kits which come with all the reagents available in the Viability PCR Starter Kits in addition to strain-specific primers.

Viability PCR Products

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
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

biotium