Viability PCR
With PMA and PMAxx™ dyes

Live cell detection and quantitation by qPCR

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-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 not only to bacteria but to other cell types as well (see page 2 for details).

v-PCR with PMA has been extensively validated
PMA from Biotium has been cited in hundreds of publications, and in many 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
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 2).

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

Viability PCR Starter Kits

The customizable Viability PCR Starter Kits are the easiest way to get started in v-PCR.

Kits contain the materials that you need 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™ qPCR Master Mix
• ROX reference dye
• 5X 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
**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)

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**Example data from the Salmonella Viability PCR Kit**

![Graph showing live and dead cells treated with PMAxx and PMAxx + Enhancer](image)

Figure 3. 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 q-PCR of mildly heat killed E. coli**

![Graph showing dCt values for live and dead cells treated with PMAxx and PMAxx + Enhancer](image)

Figure 4. 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.

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**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 4).

**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

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### Related products

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