Microbiology Enlightened

Detected dead or alive

QIAGEN®

Sample & Assay Technologies

Viability PCR for your microbiology applications

Rapid, sensitive, and specific detection of viable microorganisms is an essential requirement in many areas of microbiology research and quality control. DNA-based amplification technologies, such as real-time PCR, provide the required speed and specificity. Viability PCR represents the next advance in microbiology technologies, as it combines the speed and specificity of PCR-based methods with viability information.

Imagine all the applications that can benefit from viability PCR!

With new technologies come new possibilities. Here are just a few examples of how this exciting technology can be used.

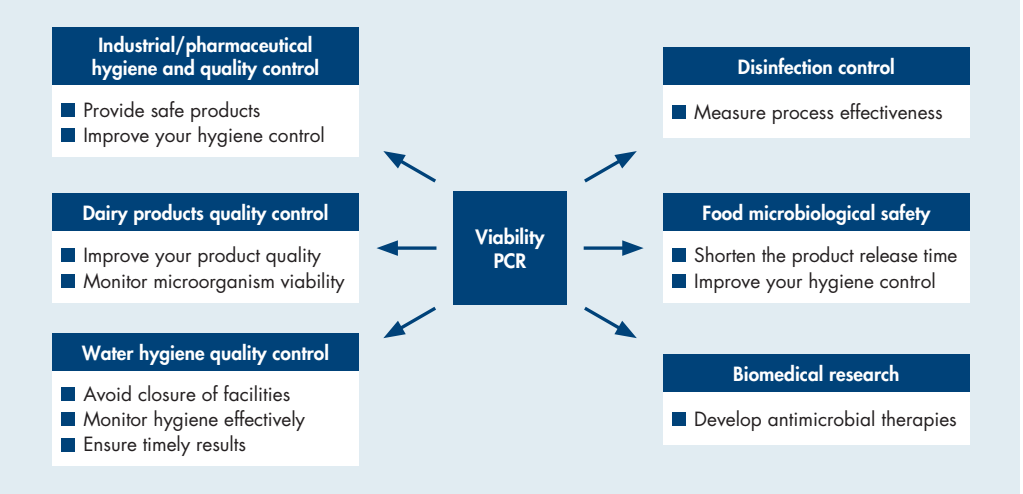


Figure 1. New possibilities using viability PCR. Viability PCR can be used in a wide variety of research and quality control applications.

Get in contact with us to discuss your ideas by mailing ViabilityPCR@qiagen.com.

How does viability PCR work?

Viability PCR is an innovative technology that enables differentiation between live and dead microorganisms. QIAGEN's viability PCR system uses the DNA-intercalating agent propidium monoazide (PMA). PMA is a chemical molecule that is applied in a constantly growing range of molecular biology applications for the differentiation of live and dead microorganisms at the DNA level. After photoactivation with a defined wavelength, PMA intercalates and binds covalently to DNA. Subsequent amplification of the modified DNA is inhibited, resulting in a reduction in the amplification signal.

PMA is unable to pass through intact biological membranes. This means that DNA from living microorganisms, where membranes are intact, is protected from PMA modification and therefore detectable by PCR. Membranes of dead microorganisms lose their protective functionality, and PMA can enter these cells and modify their DNA. The modified DNA of dead microorganisms is inhibited from detection by PCR, resulting in a reduced amplification signal.

PMA carries a strong positive charge that means it is unable to pass through intact live cell membranes. It also has an anchor group that binds irreversibly to DNA upon activation with light of a defined wavelength. These special features make PMA superior to other DNA intercalating dyes and the basis for QIAGEN's BLU-V Viability portfolio.

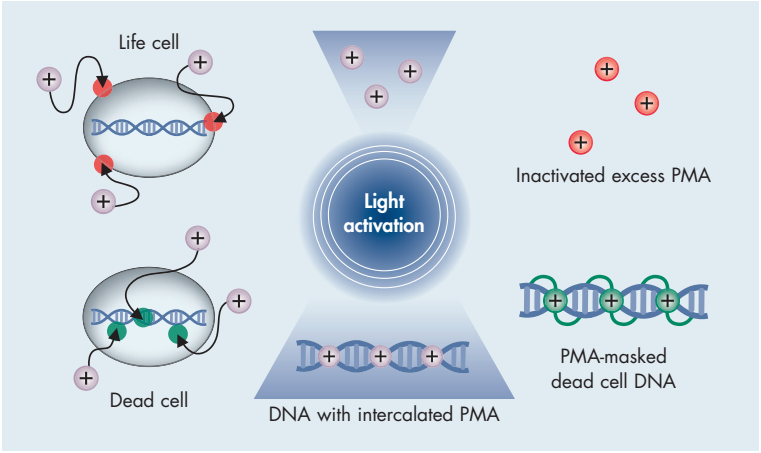
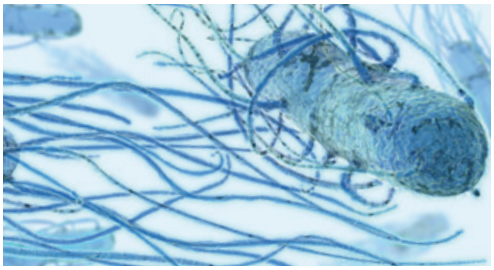


Figure 2. Principle of viability PCR. PMA cannot permeate intact membranes of living microorganisms and so downstream PCR is unaffected. Conversely, PMA is capable of permeating the membranes of dead microorganisms, followed by modification of DNA and inhibition of downstream PCR amplification.



"Viability PCR is a promising technology for microbiological live/dead differentiation, now standardized and easy-to-use for various applications with QIAGEN solutions."

Dr. Andreas Nocker-Einsiedler, developer of viability PCR using PMA technology

Viability PCR for quick, sensitive, and reliable detection of live microorganisms

Viability PCR is a novel technique that selectively amplifies the DNA of live cells. This enables researchers to quickly and reliably discriminate between live and dead cells, without relying on time-consuming, culture-based methods.

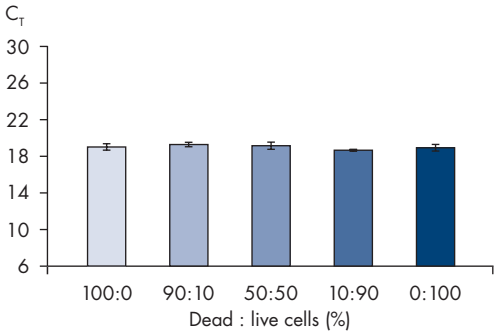


Figure 3. Conventional PCR does not differentiate between living and dead microorganisms. Mixtures of live and heat-killed salmonella were prepared. The cells were lysed and DNA was extracted. Conventional real-time PCR was then performed. No differences can be detected in the C_T values between the different cell mixtures. This demonstrates the inability of conventional PCR to distinguish between DNA from living or dead organisms.

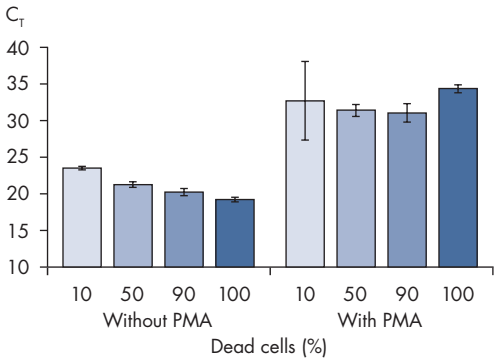


Figure 4. PMA inhibits amplification of dead cell DNA. Various concentrations of dead salmonella were prepared in buffered peptone water and processed with or without PMA treatment. There is a distinct signal shift to higher C_T values for the cells with PMA treatment compared to those without PMA treatment, with an average ΔC_T value of ~11.4. This suggests that PMA inhibits amplification of DNA of dead salmonella. A titration in the C_T values for PMA-treated salmonella corresponding to the signal titration for non-PMA treated salmonella is not observed, due to the low copy number range which is at the lower limit-of-detection for the applied mericon Salmonella spp Kit.

Drawback of conventional PCR

One of the major drawbacks of conventional PCR is that it does not help differentiate live and dead microorganisms. This could cause misleading results, such as overestimation of contamination levels. In addition, accurate quantification of only live microorganisms is not possible (Figure 3).

PMA-based viability PCR clearly differentiates live and dead cell DNA

PMA selectively enters dead cells and upon photo-activation intercalates and binds covalently to DNA, strongly inhibiting subsequent PCR amplification. The resulting C_T signal from dead cell DNA is significantly higher in comparison to live cell DNA, providing a clear distinction between live and dead cells (Figure 4).

PMA selectively suppresses detection of dead cell DNA

When applied to a sample containing mixtures of live and dead microorganisms, PMA selectively suppresses the detection of dead cells without affecting the detection of live cell DNA (Figure 5). The dead cell load in the sample does not affect the performance of PMA. So you can be confident that your results accurately reflect the live cell load present in the sample.

Highly efficient live-dead cell differentiation capacity of PMA

The live-dead cell differentiation capacity of PMA ensures you can measure the effectiveness of your disinfection control measures easily and with absolute certainty. When the C_T differences (ΔC_T) between live-dead salmonella mixtures with and without PMA were compared, a maximal signal shift of ~15 to higher C_T values between the non-PMA treated and PMA treated signals was observed. The observed signal

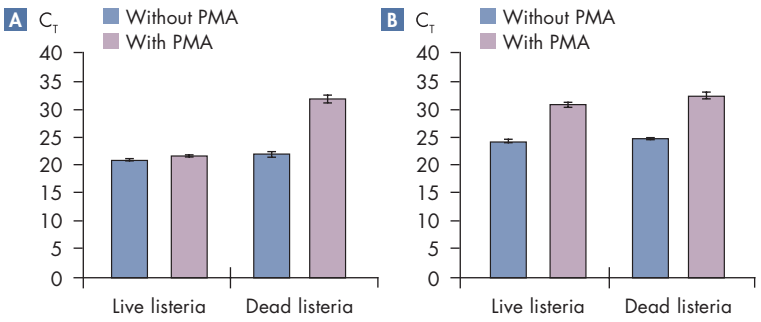
separation enables clear discrimination of PMA-bound dead cell DNA and unaltered live cell DNA. This differentiation efficiency also holds true for a small change in the live cell load of the sample (Figure 6). This ensures you can measure the effectiveness of your disinfection control measures easily and with absolute certainty.

Effective performance of PMA in a turbid matrix

One of the concerns in using a photoactivated compound, for cell viability detection, is the possibility of suboptimal performance in turbid medium. However, the wavelength of light used to activate PMA is capable of penetrating even turbid media.

To ensure correct handling and performance of the PMA reagent, a control DNA (spiked-in DNA) is included in the listeria assay set up described below. Comparison of C_T values for control DNA in PMA treated samples and untreated samples can be used to verify the correct handling and performance of the PMA reagent. Figures 7 shows the results of the PMA live/dead cell differentiation workflow carried out with detection in a target channel and also control DNA (control channel) in samples of live and heat-killed listeria. In the target channel, as expected there is no difference in the live listeria sample C_T with or without PMA. Implementation of PMA in the dead listeria samples causes the expected shift in the C_T as a consequence of successful PMA intercalation with the DNA (Figure 7A). In the control channel, DNA was spiked into the listeria samples, shows the expected C_T shift of ~6, which is still within the defined range of 6±2, regardless of the applied Nutella® matrix (Figure 7B).

The data clearly demonstrate that the intensity of the PMA activating light was sufficient enough to penetrate even a turbid sample and to lead to successful DNA intercalation.



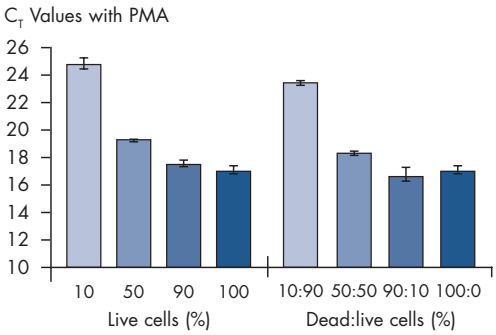


Figure 5. In live-dead cell mixtures, dead cell DNA remains selectively undetected. The bars on the left hand side represent live salmonella cells alone in buffered peptone water. The bars on the right hand side represents the corresponding mixtures containing 10:90%, 50:50%, 90:10%, and 100:0% live to heat killed ratios of salmonella bacteria. The results demonstrate that the C_T values between the two sets are highly consistent (variations below 1 C_T). This confirms that PMA is capable of suppressing the detection of dead salmonella and provides a high level of differentiation power in samples with a mixture of live and dead bacteria.

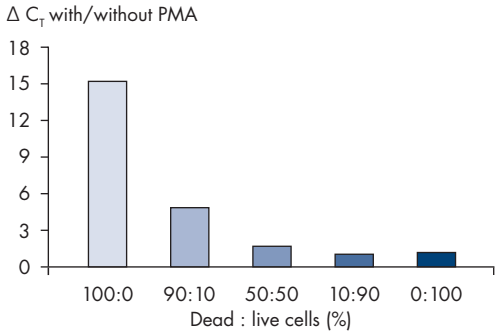


Figure 6. PMA provides highly efficient live-dead cell differentiation. C_T differences of live-dead salmonella mixtures with and without PMA are shown. The sample containing 100% dead salmonella, when treated with PMA, shows a ~15 higher C_T value when compared with its untreated counterpart. PMA-induced signal quenching is not linear, it increases in the live cell content resulting in large decreases in C_T in PMA-treated samples compared to untreated samples, thereby increasing the overall differentiation sensitivity.

Figure 7. Effect of a homogenous matrix on live/dead differentiation capabilities of PMA. A Live or heat-killed Listeria were spiked into a 2.5% Nutella matrix and processed separately. Live Listeria does not show a C_T difference when processed with or without PMA. PMA implementation on dead Listeria shows the expected C_T signal shift, indicating a successful DNA intercalation. B Control DNA was added to either live or dead cells in the same 2.5% Nutella matrix. The background of either live or dead cells exhibits no significant influence on Control DNA detection, which responds only to the presence or absence of PMA. The C_T shift of ~6 is still within the defined range of 6±2. This suggests that the intensity of the PMA activating light was high enough to penetrate a turbid sample and permit a successful DNA intercalation.

Viability PCR made easy with QIAGEN's BLU-V Portfolio

The BLU-V Viability PMA Kit, BLU-V System, and the BLU-V Incubation Box make up the BLU-V portfolio. Together, they provide a streamlined workflow to get you started with viability PCR applications.

BLU-V Viability PMA Kit

The BLU-V Viability PMA Kit is a consumables kit covering the PMA treatment, which is compatible with various QIAGEN DNA extraction kits, such as QIAamp UCP Pathogen Mini Kit, QIAamp cador Pathogen Mini Kit, DNeasy Blood & Tissue Kit, and QIAGEN real-time PCR kits.

The BLU-V Viability PMA Kit provides:

- Maximum convenience for an easy start with viability PCR
- Optimized reagents, conditions, and protocols for the PMA treatment
- High compatibility with QIAGEN DNA extraction and real-time PCR kits
- Generic setup, allowing flexible adjustments for your specific application

BLU-V System

The BLU-V System is a small benchtop instrument for standardized photoactivation of PMA simply and efficiently.

Features of the BLU-V system include:

- Simultaneous photo-activation with a defined light wavelength of up to 12 samples
- Constant and uniform light dosage for optimal PMA activation
- No heating of samples during PMA photo-activation

BLU-V Incubation Box

The BLU-V incubation box is an accessory for light-protected incubation of samples.

Viability PCR workflow

Implementation of the viability PCR workflow is straightforward and applies a trusted and simple-to-use technology for specific detection of viable microorganisms in a mixed population.

Learn more about BLU-V at www.qiagen.com/bluv.

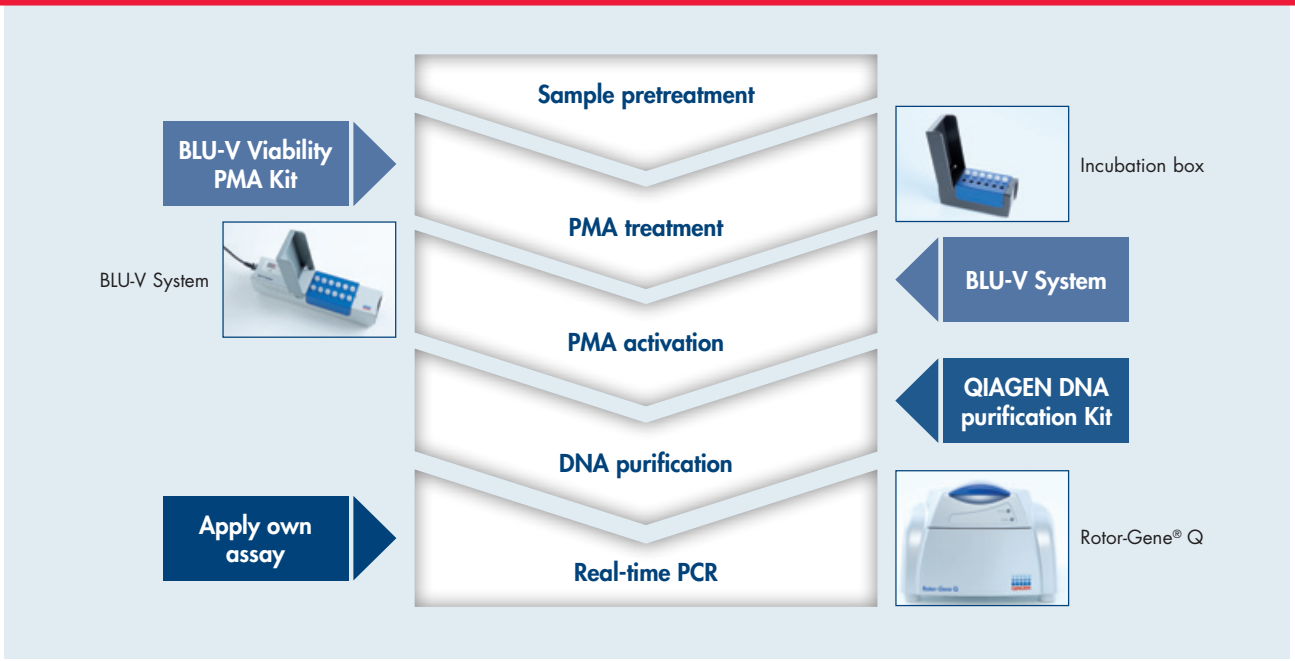


Figure 8. Viability PCR workflow. Implementation of BLU-V can be easily incorporated into your lab workflow.

Integrate viability PCR into your lab's workflow, with our BLU-V portfolio, and be confident of accurately differentiating living and dead microorganisms.

Ordering Information

Product	Contents	Cat. no.
BLU-V Viability PMA Kit	PMA Reagent 2 x 0.7 mg; Buffer EB, RNase-free water	296015
BLU-V System	Instrument for photo-activation of dyes, includes one BLU-V Sample Holder	9002300
BLU-V Incubation Box	Box for up to 12 tubes (tube volumes up to 2 ml) for light protection during incubation	9022908
BLU-V Sample Holder	Sample holder for up to 12 tubes (tube volumes up to 2 ml)	9022909
QIAamp UCP Pathogen Mini Kit	50 QIAamp UCP Mini Columns, Collection Tubes (2 ml), Tube Extenders (20 ml), Elution Tubes, VacConnectors, Buffers, and Proteinase K	50214
QIAamp *cador* Pathogen Mini Kit (50)*	For 50 RNA/DNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free buffers	54104
DNeasy Blood & Tissue Kit (4)*	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96-Well Plate Registers	69581

* Other kit sizes are available; see www.qiagen.com.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

www.qiagen.com

Australia ▪ Orders 1-800-243-800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800-28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Telephone 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325 or 400-880-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-920 or 0800-912965 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 or 0800-912961

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

India ▪ Orders 1-800-102-4114 ▪ Fax 1-800-103-4114 ▪ Technical 1-800-102-4115

Ireland ▪ Orders 1800-555-049 ▪ Fax 1800-555-048 ▪ Technical 1800-555-061

Italy ▪ Orders 800-789544 ▪ Fax 800-789660 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-436

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 1800-742-4362 ▪ Fax 65-6854-8184 ▪ Technical 1800-742-4368

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 or 0800-897470 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12 or 0800-837160

Taiwan ▪ Orders 0800-665-956 ▪ Fax 8862-2369-1100 ▪ Technical 0800-665-957

UK ▪ Orders 0808-234-3665 ▪ Fax 0808-234-3918 ▪ Technical 0808-234-3974

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)

1075650 01/2014

Sample & Assay Technologies