Selective Detection of Viable Bacteria Using PMA™ Dye in Conjunction with qPCR

The ability to selectively and sensitively detect viable bacteria in the presence of dead bacteria is of vital importance for many practical applications, including safety inspection of food products, drinking water quality control, and medical diagnosis. The traditional detection method based on bacterial culturing is time-consuming and insensitive. Detection based on PCR is a rapid and highly sensitive alternative method. However, PCR methods cannot distinguish live from dead cells, making interpretation of any analytical results difficult. The novel DNA-modifying dye Propidium monoazide (PMA™) developed by Biotium overcomes this problem. Pre-treating a sample with PMA™ prior to PCR analysis permits one to selectively detect only viable bacteria in a highly sensitive and reliable manner.

PMA™ is a photo-reactive dye with a high affinity for DNA. The dye intercalates into dsDNA and forms a covalent linkage upon exposure to intense visible light, resulting in chemically modified DNA, which cannot be amplified by PCR (Figure 1). Because PMA™ is designed to be cell membrane-impermeable, when a sample comprising both live and dead bacteria is treated with PMA™, only dead bacteria are susceptible to DNA modification due to compromised cell membranes. Thus, subsequent lysis of live bacteria followed by qPCR permits selective detection of the live cells.

The PMA™-qPCR technology can be applied not only to bacteria but to other cell types as well. Biotium also offers other complementary products, including our superior EvaGreen® qPCR dye and environmentally safe and highly sensitive GelRed™ and GelGreen™ nucleic acid gel stains.

References:

1) Kobayashi, et al. (2009) Improving clinical significance of PCR: use of propidium monoazide to distinguish viable from dead Staphylococcus aureus and Staphylococcus epidermidis. J Orthop Res 27:1243-1247.

2) Kobayashi, et al. (2010) Distinction between intact and antibiotic-inactivated bacteria by real-time PCR after treatment with propidium monoazide. J Orthop Res. In print.

3) Luo, et al. (2009) Method to detect only viable cells in microbial ecology. Appl Microbiol Biotechnol 86:377-384.

4) Nocker, et al. (2006) Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J Microbiol Methods 67:310-320.

5) Nocker, et al. (2007) Use of propidium monoazide for live/dead distinction in microbial ecology. Appl Environ Microbiol 73:5111-5117.

6) Varma, et al. (2009) Quantitative real-time PCR analysis of total and propidium monoazide-resistant fecal indicator bacteria in wastewater. Water Res 43:4790-4801.

7) Vesper, et al. (2008) Quantifying fungal viability in air and water samples using quantitative PCR after treatment with propidium monoazide (PMA). J Microbiol Methods 72:180-184.

Principle of the Technology

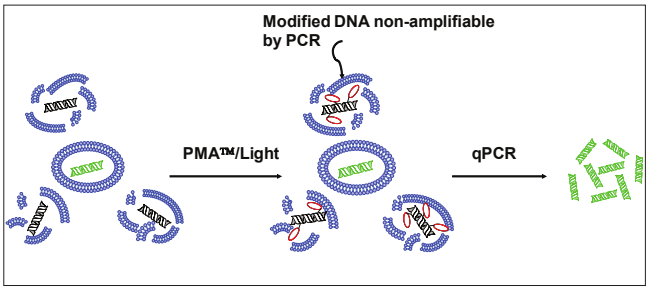
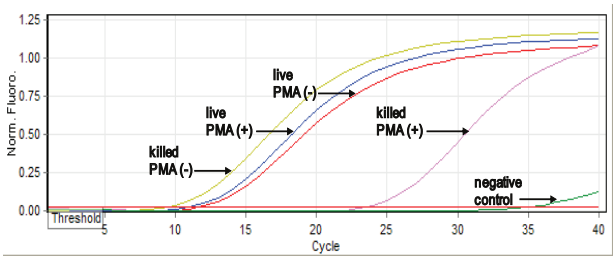


Figure 1. The cell membrane-impermeable PMA™ dye selectively and covalently modifies DNA from dead bacteria with compromised membrane while leaving DNA from viable cells intact. Because PMA™-modified DNA can not be amplified, subsequent lysis of viable cells and qPCR permit selective quantitation of viable bacteria.

A



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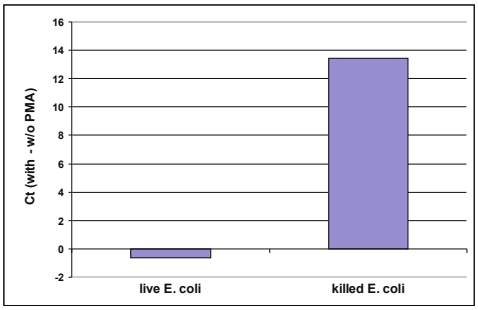

Figure 2. Effect of PMA on quantitative PCR of live and heat-inactivated E. coli. (A) A representative quantitation analysis of real-time PCR performed on live and dead E. coli treated with PMA using primers against a region of the 16S rRNA. (B) The ΔCt of live and killed E. coli with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). Both live and killed E. coli were incubated with or without PMA and exposed to light for 5 minutes.

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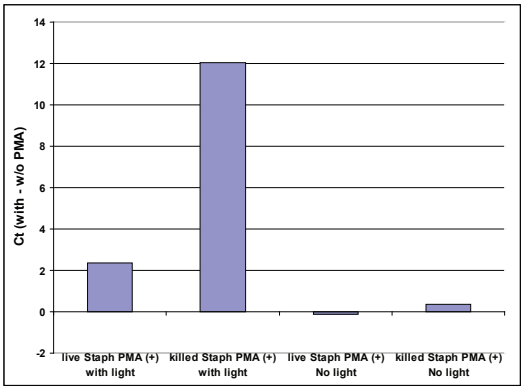

Figure 3. Effect of PMA on quantitative PCR of live and heat-inactivated Staphylococcus epidermidis. The ΔCt of live and killed S. epidermidis with and without PMA treatment in addition to live and killed bacteria mixed with PMA but without photolysis by light exposure (No light). The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). All samples were incubated with PMA for 5 minutes in the dark with occasional mixing, and then the appropriate samples were exposed to light for 5 minutes.

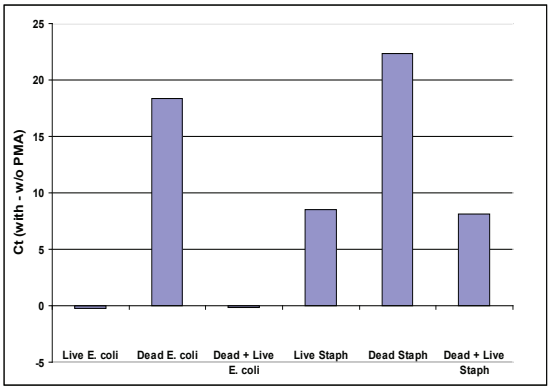

Figure 4. Mix of live and dead E. coli and S. epidermidis. The ΔCt of live, killed, and a mix of live/killed bacteria with and without PMA treatment. The Ct value of sample without PMA was subtracted from the corresponding sample with PMA cross-linking (Ct with PMA – Ct without PMA). Amplification was for a region of the 16S rRNA. All samples were incubated with PMA for 5 minutes, and then exposed to light for 5 minutes.

Basic Protocol for PMA™-qPCR Technology

- Add PMA™ dye to your sample and incubate in the dark.
- Cross-link PMA™ dye to DNA by exposing to light.
- Lyse intact cells and extract DNA using method of your choice.
- Perform conventional or real-time PCR.

Table 1. PMA™ and related products

Cat. #	Product Name	Unit Size	Product Feature
40013	PMA™ dye	1 mg	Supplied as lyophilized powder, convenient for long-term storage.
New! 40019	PMA™ dye in H_2O, 20 mM	100 µL	No more DMSO for stock solution preparation to minimize dye diffusion into live cells.
Other Related Products			
New! 31014	Fast EvaGreen® Master Mix for qPCR and HRM with Rox	200 rxn	Suitable for both qPCR and HRM, the products comprise novel intercalating EvaGreen® dye and our superior chemically-modified hotstart Cheetah™ Taq to deliver unmatched results. Select #31015 for ABI 7700 and 7900 instruments. For other qPCR instruments, select #31014.
New! 31015	Fast EvaGreen® Master Mix for qPCR and HRM with high Rox	200 rxn	
New! 31016	Fast Probe Master Mix with Rox	200 rxn	Comprises our fast-activating hotstart Cheetah™ Taq and proprietary buffer. Suitable for use with TaqMan®-like probes.
41003	GelRed™ nucleic acid gel stain, 10,000X in H_2O	0.5 mL	Environmentally safe and ultra sensitive nucleic acid gel stain. Perfect replacement for EtBr.
New! 31010	GelRed™ nucleic acid gel prestaining kit (sufficient for 100 mini gels)	each	The safest and fastest way to stain gels. The kit includes loading buffer containing GelRed™ dye and tracking dyes, and a 200X running buffer compatible with high-voltage, fast electrophoresis.
41005	GelGreen™ nucleic acid gel stain, 10,000X in H_2O	0.5 mL	Environmentally safe and ultra sensitive nucleic acid gel stain. Excitable by UV or visible light. Perfect replacement for EtBr or SYBR® Safe.