SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Revised: June 24, 2022

Product Information

Strain-Specific Bacterial Viability PCR Kits

Kit Contents

Component	Kit Containing	
	PMA	PMAxx™
40019: PMA Dye, 20 mM in H_2O	1 x 100 uL	-
40069: PMAxx™ Dye, 20 mM in H_2O (included in the following kits: 31033-X, 31037-X, 31050-X, 31051-X)	-	1 x 100 uL
31038: PMA Enhancer for Gram Negative Bacteria, 5X Solution (included in the following kits: 31033, 31033-X, 31037, 31037-X, 31050, 31050-X, 31053)	1 x 16 mL	1 x 16 mL
31042C: ROX Reference Dye	1 x 1 mL	1 x 1 mL
99801: Forget-Me-Not™ EvaGreen® qPCR Master Mix (2X)	2 x 1 mL	2 x 1 mL
Primer mix, 5 uM each primer	1 x 400 uL	1 x 400 uL

Unit Size: 1 kit (200 PCR reactions)

Storage and Handling

Store kit at -20°C. PMA Enhancer, if included in the kit, should be stored at 4°C after first thaw. Store PMA, PMAxx™, and Forget-Me-Not™ EvaGreen® qPCR Master Mix protected from light. Protect PMA and PMAxx™ from light during use. Components are stable for at least 6 months when stored as recommended. Kit components are stable for several freeze/thaw cycles.

Spectral Properties

PMA and PMAxx™: λ_{abs} = 464 nm (before photolysis)
$\lambda_{abs}/\lambda_{em}$ = ~510/~610 nm (following photolysis and reaction with DNA/RNA)

EvaGreen®: λ_{abs} = 471 nm (without DNA)
$\lambda_{abs}/\lambda_{em}$ = 500/530 nm (with DNA)

Product Description

Viability PCR kits are designed for selective detection of viable bacteria by qPCR. Each kit contains a viability dye (PMA or PMAxx™), Forget-Me-Not™ EvaGreen® qPCR Master Mix, and PCR primers for detection of a specific strain of bacteria (see Table 1). See Table 6 on page 5 for information on each strain.

This kit contains reagents sufficient to treat approximately 80 bacterial samples with PMA or PMAxx™ dye or 200 PCR reactions. The optimal amount of dye used and the number of samples that can be treated may vary with sample type.

PMA and PMAxx™ are photoreactive DNA binding dyes developed by Biotium. They are cell membrane-impermeant and selectively bind to DNA from dead cells with compromised membrane integrity, while leaving DNA from viable cells intact. Upon photolysis, the dyes form a stable covalent bond, resulting in permanent DNA modification. The modifications inhibit PCR amplification of DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases (1). Thus the dyes are useful in the selective detection of viable cells by real-time qPCR (Figure 1).

Some strain-specific bacterial viability PCR Kits contain PMA Enhancer for Gram Negative Bacteria. This solution is designed for use with both PMA and PMAxx™. When PMA Enhancer is added to gram-negative bacteria before treatment with viability PCR dye, dead cell DNA levels are further decreased, and thus live-dead cell discrimination is improved. The amount of improvement varies between bacterial strains and the way that the bacteria were killed. PMA Enhancer gives the most improvement when bacteria are dead but their membranes are not completely disrupted, which can occur after mild heat treatment. PMA Enhancer should never be used with gram-positive bacteria.

Forget-Me-Not™ EvaGreen® qPCR Master Mix is a hot-start EvaGreen® Dye-based master mix for use in qPCR applications and DNA melt curve analysis. Forget-Me-Not™ EvaGreen® qPCR Master Mix contains a low concentration of blue dye, which allows you to see at a glance whether you forgot to add master mix to any of your tubes, so you can catch pipetting mistakes and avoid wasting time, reagents, and your precious DNA samples. It is formulated for qPCR using a fast cycling protocol but can also be used for qPCR using regular cycling protocols. Forget-Me-Not™ EvaGreen® qPCR Master Mix contains Cheetah™ Taq, Biotium's fast-activating chemically-modified hot-start Taq polymerase, which is ideal for fast PCR cycling protocols.

Table 1. Kit Information by Strain

Bacterial strain	Gene name	Catalog number			Primer sequences	Extension temperature	Primer reference
		PMA	PMAxx™	PMA Enhancer			
Salmonella enterica	invA	31033	31033-X	31033, 31033-X	For: 5'-ATTCTGGTACTAATGGTGATGATC-3' Rev: 5'-GCCAGGCTATCGCCAATAAC-3'	60°C	Internal data
Mycobacterium tuberculosis	groEL2	31034	-	-	For: 5'-CTAGGTCGGGACGGTGAGGCCAGG-3' Rev: 5'-CATTGCGAAGTGATTCCTCCGGAT-3'	55°C	Pao C. C., et al. (see Ref. 2)
Staphylococcus aureus	nuc	31035	-	-	For: 5'-GCGATTGATGGTGATACGGTT-3' Rev: 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	52°C	Fang H. and Hedin G. (see Ref. 3)
Staphylococcus aureus	mecA	31036	-	-	For: 5'-GCAATCGCTAAAGAACTAAG-3' Rev: 5'-GGGACCAACATAACCTAATA-3'	52°C	Fang H. and Hedin G. (see Ref. 3)
E. coli O157:H7	Z3276	31037	31037-X	31037, 31037-X	For: 5'-GCACTAAAAGCTTGGAGCAGTTC-3' Rev: 5'-AACAATGGGTCAGCGGTAAGGCTA-3'	52°C	Li B. and Chen J. (see Ref. 4)
E. coli	uidA	31050	31050-X	31050, 31050-X	For: 5'-TGGATCGCGAAAACTGTGGA-3' Rev: 5'-CGGTGATATCGTCCACCCAG-3'	64°C	Internal data
Listeria monocytogenes	hly	31051	31051-X	-	For: 5'-GGGAAATCTGTCTCAGGTGATGT-3' Rev: 5'-CGATGATTTGAACTTCATCTTTTGC-3'	63°C	Internal data
Legionella pneumophila	mip	31053	-	31053	For: 5'-GCAATGTCAACAGCAA-3' Rev: 5'-CATAGCGTCTTGCATG-3'	52°C	Wilson DA., et al. (see Ref. 5)

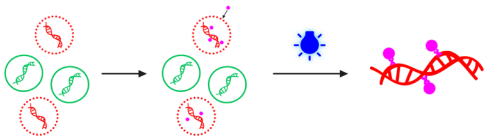


Figure 1. Viability PCR dyes like PMAxx™ or PMA are membrane-impermeant, which makes them dead cell specific. Once inside of a dead cell, they bind to DNA. Exposure to intense visible light renders the dyes reactive, and causes them to covalently attach to the DNA. This DNA modification prevents amplification in subsequent PCR reactions.

References

PMA and PMAxx™ from Biotium have been cited in hundreds of publications. View PMA and PMAxx™ References for an updated list of selected references.

1) Nocker A., et al. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbiol. Meth. 67(2), 310-320 (2006).

2) Pao C. C., et al. Detection and identification of Mycobacterium tuberculosis by DNA amplification. J. Clin. Microbiol. 28(9), 1877-80 (1990).

3) Fang H. and Hedin G. Rapid screening and identification of methicillin-resistant Staphylococcus aureus from clinical samples by selective-broth and real-time PCR assay. J. Clin. Microbiol. 41(7), 2894-9 (2003).

4) Li B. and Chen J. Real-time PCR methodology for selective detection of viable Escherichia coli O157:H7 cells by targeting Z3276 as a genetic marker. Appl Environ Microbiol. 78(15):5297-304 (2012).

5) Wilson D. A., et al. Detection of Legionella pneumophila by real-time PCR for the mip gene. J. Clin. Microbiol. 41 (7) 3327-3330 (2003).

Considerations for viability PCR

1. Viability PCR differentiates viable from non-viable cells based on cell membrane permeability. Many methods of killing cells result in compromised cell membranes, and are thus compatible with viability PCR. However, some methods, such as UV light exposure, may not immediately result in disrupted cell membranes. Literature searches and pilot studies may help to determine whether viability PCR will work with your chosen cell type and killing method.
2. It is advisable to choose the appropriate dye for your viability PCR experiment. Biotium offers three different viability PCR dyes: EMA, PMA, and PMAxx™. In general, we recommend using PMAxx™ for bacterial samples and PMA for yeast and fungal samples. EMA is more permeant to live cells and thus often gives false negative results. However, you may want to test more than one dye to determine the optimal dye for your sample type.
3. Freezing of samples prior to performing viability PCR may damage the cell membrane and give false negative results. A pilot test is recommend before attempting to freeze your samples prior to dye treatment. Samples can be frozen after dye treatment and photolysis.
4. If your sample of interest is a gram-negative bacterium, you may want to use PMA Enhancer for Gram Negative Bacteria (Cat. no. 31038). This product is added to the sample during the viability PCR process, and improves the discrimination between live and dead cells. However, if your sample is a gram-positive strain, or a mixture of gram-negative and gram-positive, the Enhancer should not be used.
5. Viability PCR requires a photoactivation step in order for the dye to covalently bind to the dead cell DNA. The PMA-Lite™ LED Photolysis Device (Cat. no. E90002), designed for use with 1.5 mL tubes, is the most efficient device for performing this function. Other blue or white light sources may also be used. In general, the brighter the lights, the more efficiently they will perform the photolysis step. Non-LED lights, such as halogen lamps, may heat your sample and negatively affect the assay.
6. Part of the proposed mechanism of action of PMA and PMAxx™ is the removal of PMA or PMAxx™-bound DNA from samples via precipitation; therefore the amount of template DNA in each qPCR reaction should not be normalized between samples. Instead, PCR should be performed using equal volumes of gDNA eluate from each sample. As a positive control for the qPCR reaction, 1 ng of purified genomic DNA (gDNA) should be sufficient for achieving good signal.
7. In order to validate PMA or PMAxx™ effectiveness in your sample of interest, we recommend utilizing live-cell and dead-cell controls, each with and without PMA or PMAxx™ (Figure 3). The change in Ct (dCt) caused by PMA or PMAxx™ for each control should be assessed (see “Data analysis”, page 4).
8. Treatment of complex samples, such as feces or soil, may require optimization of sample dilution, dye concentration, and light treatment time.
9. Treatment of dilute samples, such as water testing, may require filtration or concentration before PMA or PMAxx™ treatment.

Before you begin

- Read the “Considerations for viability PCR” section above to determine the appropriate viability dye, primers, Enhancer, and light source to use in your experiment.
- Ensure that you have a workspace that is protected from direct light. The steps of the protocol that require opening the vial of viability dye, pipetting viability dye, and incubating with viability dye should be done in in dim light.

Table 2. Recommended ROX Concentration for PCR Instruments

PCR instrument	Recommended ROX concentration	Size amount of 10X ROX per 20 uL reaction
BioRad: iCycler™, MyiQ™, MyiQ™ 2, iQ™ 5, CFX-96 Touch™, CFX-384 Touch™, MJ Opticon, Option2, Chromo4, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene®3000, Rotor-Gene® 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCyler® Roche: LightCycler® 480, LightCycler® 2.0	No ROX	None
Applied Biosystems: 7500, 7500 Fast, ViiA™ 7 Stratagene: MX4000P, MX3000P, MX3005P	Low ROX	Dilute ROX 1:100 with dH2O and add 3 uL of diluted ROX per 20 uL reaction.
Applied Biosystems: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX	Dilute ROX 1:10 with dH2O and add 3 uL of ROX Reference Dye per 20 uL reaction.

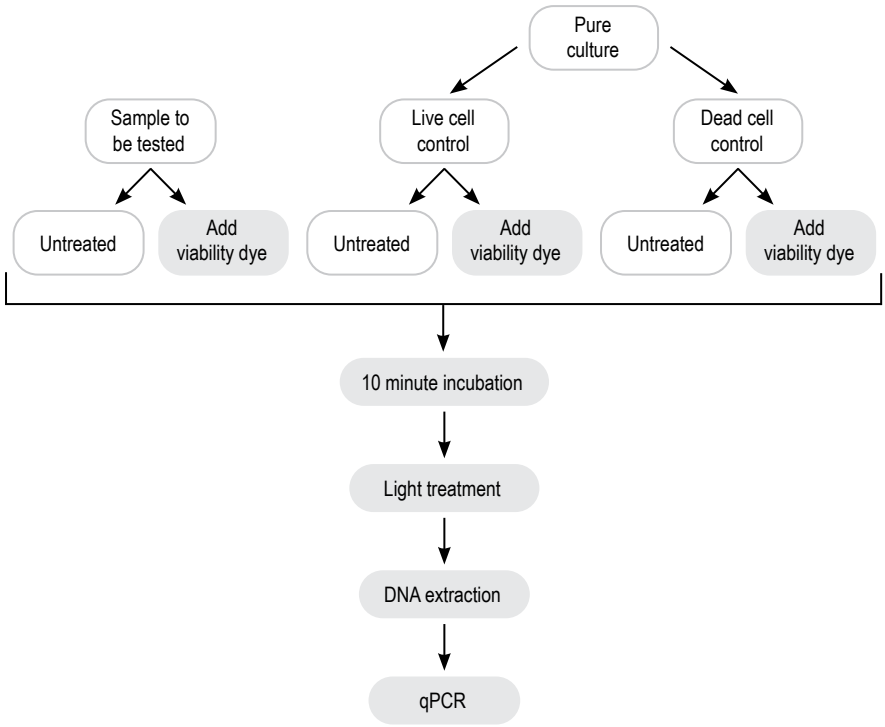


Figure 2. Viability PCR workflow overview, with recommended live and dead cell controls.

Quick guide protocol

1. Aliquot 400 uL of cell culture or sample into tubes. If desired, prepare live and dead cell controls.
2. If PMA Enhancer for Gram Negative Bacteria is included in this kit, add 100 uL of 5X PMA Enhancer to a final concentration of 1X. See detailed protocol below for more information.
3. Working in dim light, add PMA or PMAxx™ to samples at a final concentration of 25-50 uM. Include no-dye controls.
4. Incubate for 10 minutes, rocking, protected from light.
5. Expose samples to light to crosslink dye to DNA. We recommend 15 minutes in the PMA-Lite™ LED Photolysis Device (Cat. no. E90002).
6. Isolate DNA using a commercial kit or other protocol.
7. Set up qPCR reactions, using 2 uL of each isolated DNA sample as templates. Do not normalize the DNA concentrations.
8. Compare the amount of total and live cell-derived DNA in your sample by calculating the dCt (dCt = Ct with viability dye - Ct without viability dye). See “Data analysis” on page 4 for more information.

Detailed protocol for treating bacteria with PMA or PMAxx™ for qPCR

See Figure 2 for an overview of the procedure. This is a general protocol for treating cultured strains of bacteria with PMA or PMAxx™. It is recommended that you first perform a control experiment with live and dead controls for your selected organism.

1. Inoculate your desired media broth with bacteria (volume is dependent on size of experiment). Culture bacteria overnight or longer on a shaker at 200 rpm overnight at 37°C until the OD_{600} of the culture is approximately 1.
2. Suggested: To prepare dead cell control samples, heat inactivate bacteria at 95°C for 5 min. If it is desired to compare viability PCR with plate-based viability, plate 10 uL of heat inactivated bacteria on the appropriate media plate, and 10 uL of a 1:100 dilution of control bacteria on another plate. Grow on plates at optimal growth temperature and check for colony growth after 24-48 hours.

   Note: For *Salmonella enterica* and *Legionella pneumophila*, dead cell control samples may also be heated for 58°C for 3 hours.
3. Pipette 400 uL aliquots of bacterial culture into clear microcentrifuge tubes. For each sample you will need one tube for PMA or PMAxx™-treated cells and one tube for untreated (no dye added) cells in order to calculate dCt (see Figure 2, “Considerations for viability PCR” #7, and “Data analysis” for additonal details).
4. Optional: If kit includes PMA Enhancer, add 100 uL of 5X Enhancer to each tube for a 1X final Enhancer concentration (see “Considerations for viability PCR” #4).
5. Working in low light, prepare a working stock of PMA or PMAxx™ by diluting the 20 mM dye to 5 mM in water. Add viability dye to each sample tube according to the Table 3 below:

Table 3. Dye Treatment Recommendations

Treatment	Amount of 5 mM dye to add	Sample volume	Final dye concentration
PMA or PMAxx™ with Enhancer	2.5 uL	500 uL (400 uL sample + 100 uL Enhancer)	25 uM
PMA without Enhancer	4 uL	400 uL	50 uM
PMAxx™ without Enhancer	2 uL	400 uL	25 uM

6. Incubate tubes in the dark for 10 minutes at room temperature. Perform incubation on a rocker with tubes covered with foil for optimal mixing.
7. Expose samples to light to cross-link PMA or PMAxx™ to DNA.

   Notes:

   a. For best results, we recommend that the photo-crosslinking be carried out on Biotium’s PMA-Lite™ LED Photolysis Device (Cat. no. E90002). A 15 min exposure duration should be sufficient for complete PMA or PMAxx™ activation.

   b. Commercial halogen lamps (>600 W) for home use have been employed for photoactivating PMA in some publications, though results may be less consistent due to inevitable variation in the set-up configurations. If you use a halogen lamp, we recommend that you lay tubes on a block of ice set 20 cm from the light source. The ice block should be in a clear

tray with a piece of aluminum foil under the clear tray to reflect the light upward. Set the lamp so that the light source is pointing directly down onto the samples. Expose samples to light for 5-15 min.

8. Pellet cells by centrifuging at 5,000 x g for 10 minutes.
9. Extract genomic DNA using your desired protocol or commercially available kit for your sample type.
10. Perform qPCR using the primers included with this kit for the bacterial strain.

    Note: Part of the proposed mechanism of action of PMA and PMAxx™ is the removal of PMA-bound DNA from samples via precipitation; therefore, the amount of input DNA in each sample should not be normalized between samples. Instead, PCR should be performed using equal volumes of gDNA eluate from each sample. For a positive control, 1 ng of live cell gDNA per reaction should be sufficient for achieving good signal. For gDNA extracted from bacterial cultures using a commercial extraction kit, 2 uL of eluted DNA can be used as a starting point for optimization.

Table 4. PCR Reaction Setup

Add reaction components to each PCR tube or well according to the table below:

Reaction component	Amount per 20 uL reaction	Final concentration
2X Forget-Me-Not™ Master Mix	10 uL	1X
Primer mix, 5 uM	2 uL	0.5 uM each
Template	x uL See Note 1	See Note in Step 10
ROX	Optional	See Table 2
dH_2O	Add to 20 uL	-

Note 1: Template volume should not exceed 10% of final reaction volume.

Table 5. Fast-Cycling Parameters for qPCR on gDNA

Hold	
95°C for 2-10 minutes (see Note 1)	
Cycling	
95°C for 5 seconds	Cycle 40 times
52-64°C for 30 seconds (acquire data), see Note 2 or Table 1 for recommended extension temperatures by strain	
Melt	
57°C to 99°C	

Note 1: Activation of Cheetah™ Taq DNA Polymerase requires only 2 minutes at 95°C, but genomic DNA can take longer to denature. If you observe high background fluorescence during initial amplification cycles, increase the hold time.

Note 2: Recommended extension temperatures by bacterial strain:

- 52°C: *Staphylococcus aureus* (nuc), *Staphylococcus aureus* (mecA), *E. coli* O157:H7 (Z3276), *Legionella pneumophila* (mip)
- 55°C: *Mycobacterium tuberculosis* (groEL2)
- 60°C: *Salmonella enterica* (invA)
- 63°C: *Listeria monocytogenes* (hly)
- 64°C: *E. coli* (uidA)

11. (Optional): If it is desired to determine the absolute number of viable cells in your sample, you should also include, as templates in the qPCR reaction, genomic DNA from your cell type of known cell number (See "Calculating the absolute number of viable cells").

Data analysis

This section describes how to use the live and dead cell controls to determine whether your experiment worked, and how to calculate the percentage of live cells in your sample. It is advisable to validate your primers and PCR set-up with genomic DNA from the same cell type before beginning your viability PCR experiment.

Live and dead cell control dCt determination

1. After the qPCR run, use the instrument software to determine the threshold cycle (Ct) for each of your samples.
2. In order to determine whether PMA or PMAxx™ adequately inhibited amplification of dead cell DNA, calculate the delta Ct (dCt) for each of your control cell populations as shown:

$$dCt_{live} = Ct_{(live,\ sample,dye\text{-}treated)} - Ct_{(live,\ untreated)}$$
$$dCt_{dead} = Ct_{(dead,\ sample,dye\text{-}treated)} - Ct_{(dead,\ untreated)}$$

3. The expected result for the live cell control is a dCt close to 0 (+/- 1) (Figure 6). This indicates that PMA or PMAxx™ treatment did not affect viable cell DNA amplification. If a larger dCt is seen for the live cell control, see Troubleshooting, page 8.
4. The expected result for the dead cell control is a dCt > 4 (Figure 6). (Since Ct values are on a $\log_2$ scale, a dCt of 4 represents a ~ 16-fold decrease, or 94% of dead cell DNA removed. A dCt of 8 represents a ~ 250-fold decrease, or 99.6% of dead cell DNA removed). If a low dCt is seen for the dead cell control, see Troubleshooting, page 8.
5. The dead cell dCt that you obtain will depend on many factors, including: the bacterial strain or other cell type; how the cells were killed; the concentration of viability dye used; the amplicon length; whether Enhancer was used. We have found the following ranges:

   a. PMA: At 25 uM PMA, the dCt ranges from ~5 to 9, depending on the bacterial strain. In yeast, we have obtained dCts of 5, 7, and 10 for 50, 100, and 200 uM PMA, respectively.

   b. PMAxx™: At 25 uM, the dCt ranges from ~9 to 13, depending on the bacterial strain. In yeast, we have obtained a dCt of 7 for 100 uM PMAxx™.

Calculating the percentage of viable cells

If your live and dead cell controls look good, you can move on to determining the percentage of viable cells in your unknown samples.

1. Calculate the dCt for the unknown samples as shown:

$$dCt_{sample} = Ct_{(sample,\ sample,dye\text{-}treated)} - Ct_{(sample,\ untreated)}$$

2. You can convert the dCt into a percentage of viable cells as shown:

$$\text{Fold reduced by sample, dye-treated} = 2^{(\text{sample dCt})}$$
$$\text{\% viable} = 100 / \text{Fold reduced}$$

Calculating the absolute number of viable cells

If you want to calculate the absolute number of viable cells in your sample*, then in the same experiment you will need to run a standard curve using genomic DNA from your cell type of interest, from a known cell number. It is advisable that you have several gDNA dilutions which fall within the predetermined linear range of your qPCR assay.

1. Using the genomic DNA samples that fall within the linear range of the qPCR assay, plot a graph of Ct (y-axis) vs cell number (x-axis). Use graphing software to calculate the R^2 value (to determine linearity of the assay), slope, and y-intercept of the line.
2. Calculate the copy number of your unknown sample as shown:

$$Ct = \text{slope}(\text{cell \#}) + \text{y-intercept} \quad (y = mx + b)$$
$$\text{Cell number}_{sample} = (Ct - \text{y-intercept}) / \text{slope}$$

*This assumes that none of the viable cell DNA has been lost during the DNA purification process.

Table 6. Strain Information

Bacterial stain	Description
Salmonella enterica (invA)	*Salmonella enterica* is a gram-negative bacteria that causes the foodborne illness salmonellosis. PMA-based viability PCR for *Salmonella enterica* has been reported using the primers provided in the kit, and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4A and 5A). An example of PMAxx™-PCR using *Salmonella* is shown in Figure 3A.
Mycobacterium tuberculosis (groEL2)	*Mycobacterium tuberculosis* and *Mycobacterium bovis* are pathogenic bacteria that can infect the lungs and causes the disease tuberculosis. PCR to detect *Mycobacterium tuberculosis* has been reported using the primers provided in the kit, and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4B and 5B). Note: groEL2 primers also amplify other mycobacteria species, but products may be distinguishable by melt curve analysis.
Staphylococcus aureus (nuc)	*Staphylococcus aureus* is a gram-positive bacteria that can infect the skin and cause a variety of illnesses ranging from mild to severe. PCR specific for *Staphylococcus aureus* nuc gene has been reported using the primers provided in the kit, and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4C and 5C).
Staphylococcus aureus (mecA)	Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* bacteria that has developed antibiotic resistance and can cause difficult-to-treat infections. PCR specific for MRSA mecA gene has been reported using the primers provided in the kit, and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4D and 5D).
E. coli O157:H7 (Z3276)	*E. coli* O157:H7 is a pathogenic strain of *E. coli* bacteria. PMA-based viability PCR specific for the *E. coli* O157:H7 Z3276 gene has been reported using the primers provided in the kit, and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4E and 5E).
E. coli (uidA)	*Escherichia coli* is a commonly used laboratory bacteria, and some strains can cause digestive illness. PCR to amplify the gene uidA has been published and shown to be highly specific for *E. coli*. The primers provided in the kit have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4F and 5F). An example of PMAxx™-PCR in *E. coli* is shown in Figure 3C.
Listeria monocytogenes (hly)	*Listeria monocytogenes* is a food-borne pathogen that can cause digestive illness and fetal harm. The gene hly encodes the Listeria Lysin O (LLO) protein that is specific for *Listeria*. PMA-PCR using these primers for viability PCR in *Listeria* has been published and these primers have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4G and 5G). An example of PMAxx™-PCR using *Listeria* is shown in Figure 3B.
Legionella pneumophila (mip)	*Legionella pneumophila* is is a pathogenic species of gram-negative bacteria. It can infect the lungs and cause Legionnaire's Disease. *Legionella pneumophila* is sometimes detected in water sources such as cooling towers and swimming pools. PCR to amplify the gene mip has been published and shown to be highly specific for *Legionella pneumophila*. The primers provided in the kit have been validated at Biotium for qPCR using EvaGreen® Master Mixes (Figures 4H and 5H).

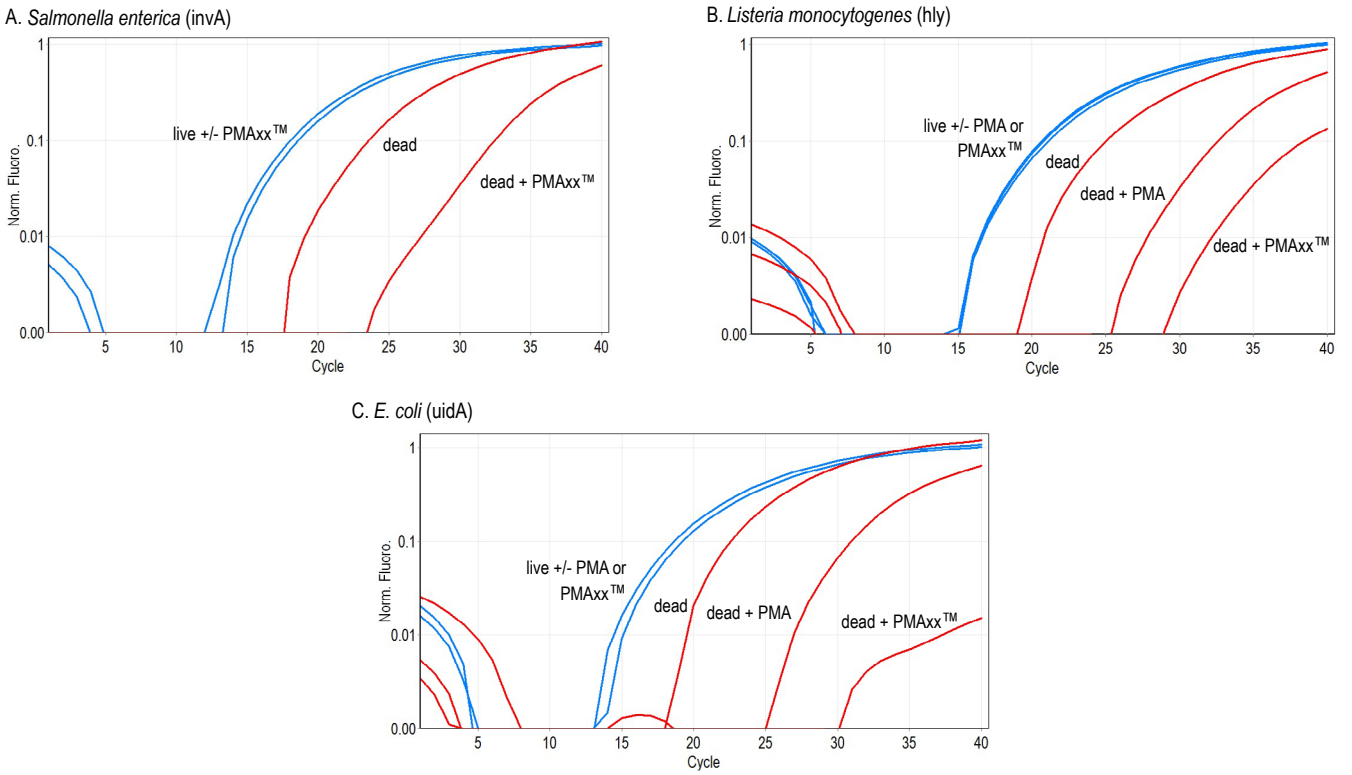
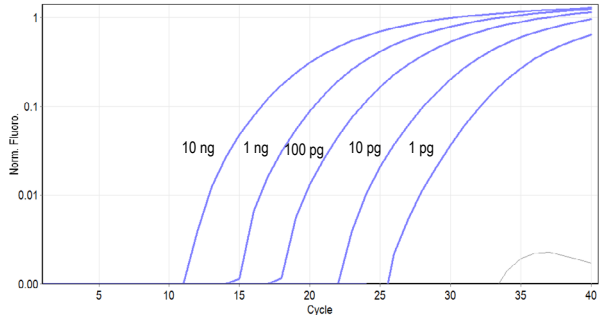
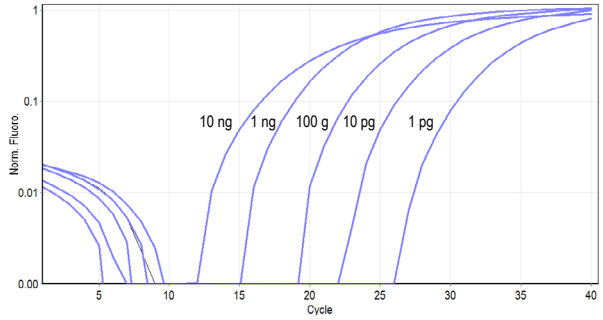


Figure 3. Live and heat-killed *Samononella enterica*, *Listeria monocytogenes*, and *E. coli* were treated with 25 uM PMAxx™, followed by photoactivation. qPCR was performed on a Qiagen Rotor-Gene® Q using gDNA from each strain with the respective A) invA, B) hly, or C) uidA primers.

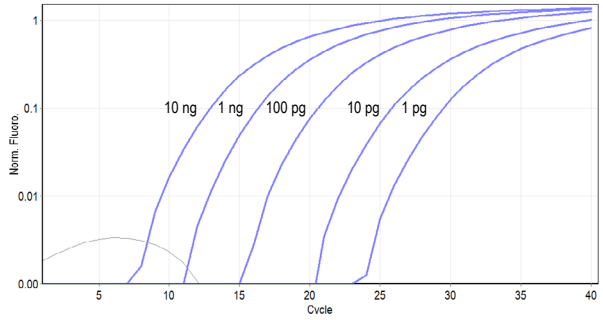
A. *Salmonella enterica* (invA)



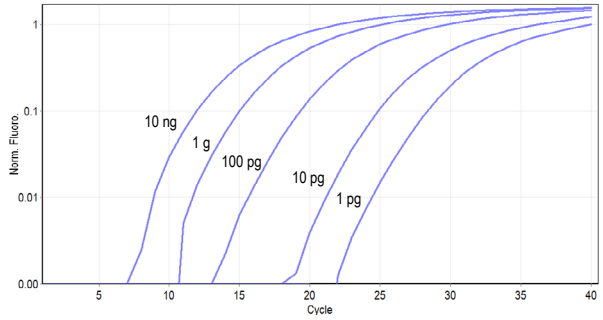
B. *Mycobacterium tuberculosis* (groEL2)



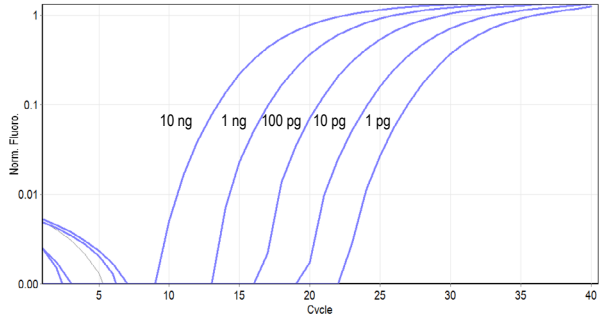
C. *Staphylococcus aureus* (nuc)



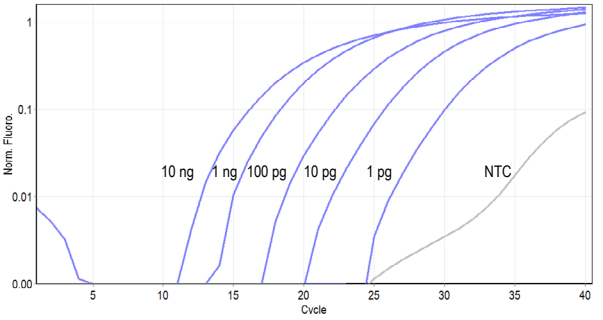
D. *Staphylococcus aureus* (mecA)



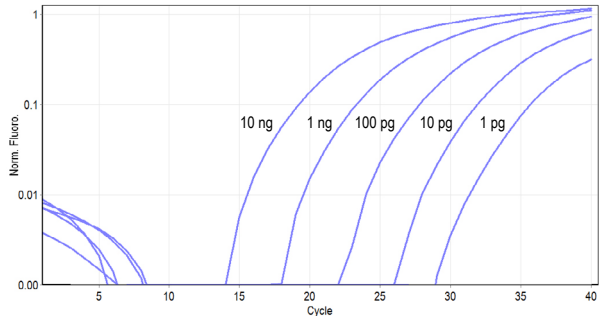
E. *E. coli* O157:H7 (Z3276)



F. *E. coli* (uidA)



G. *Listeria monocytogenes* (hly)



H. *Legionella pneumophila* (mip)

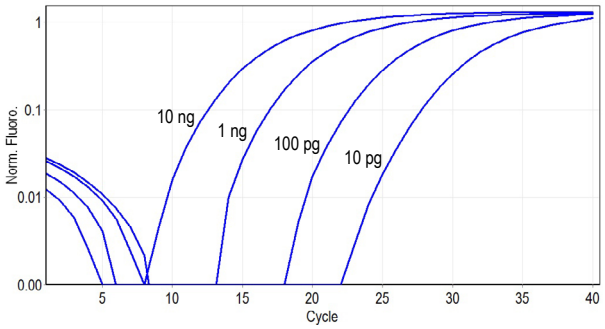
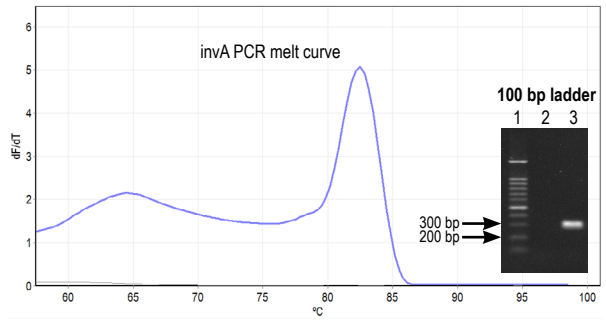
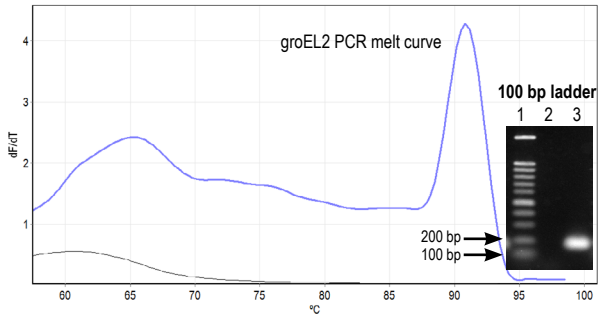


Figure 4. qPCR was performed to amplify a fragment of bacterial DNA from 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, or no-template control (NTC) of A) *Salmonella enterica* (invA), B) *Mycobacterium tuberculosis* (groEL2), C) *Staphylococcus aureus* (nuc), D) *Staphylococcus aureus* (mecA), E) *E. coli* O157:H7 (Z3276), F) *E. coli* (uidA), G) *Listeria monocytogenes* (hly), or H) *Legionella pneumophila* (mip). The qPCR was performed on a Qiagen Rotor-Gene® Q.

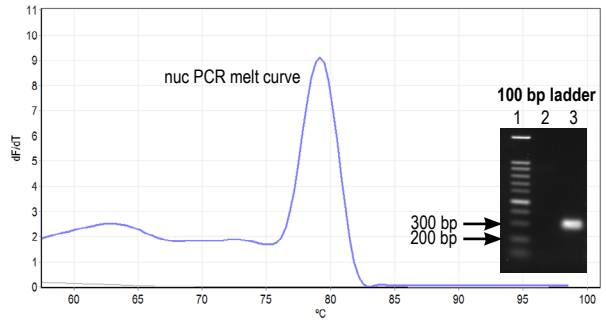
A. *Salmonella enterica* (invA)



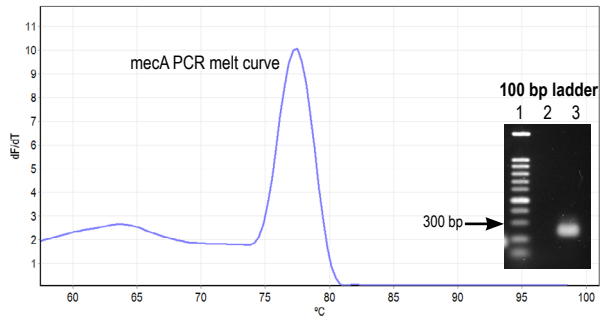
B. *Mycobacterium tuberculosis* (groEL2)



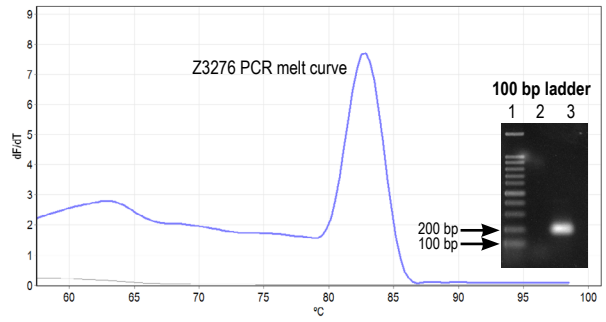
C. *Staphylococcus aureus* (nuc)



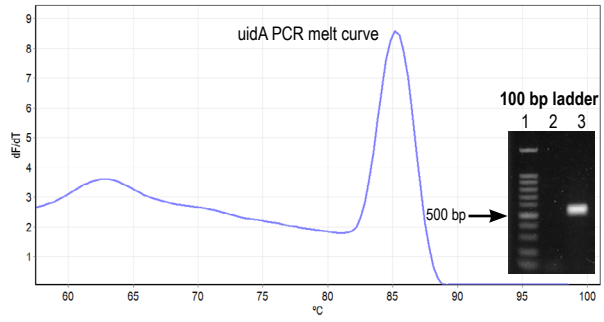
D. *Staphylococcus aureus* (mecA)



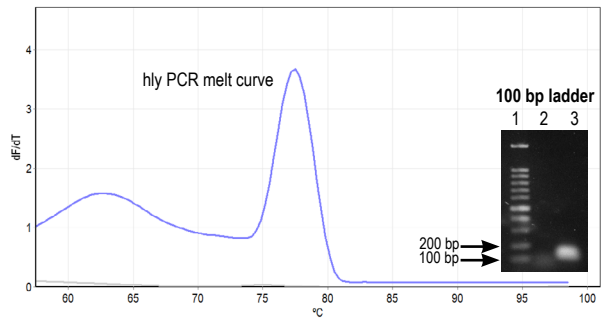
E. *E. coli* O157:H7 (Z3276)



F. *E. coli* (uidA)



G. *Listeria monocytogenes* (hly)



H. *Legionella pneumophila* (mip)

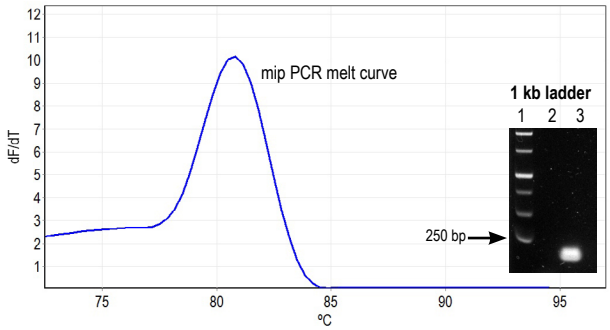


Figure 5. Melt curve analysis of the bacterial gene qPCR product generated in Figure 4, from 1 ng of A) *Salmonella enterica* (invA), B) *Mycobacterium tuberculosis* (groEL2), C) *Staphylococcus aureus* (nuc), D) *Staphylococcus aureus* (mecA), E) *E. coli* O157:H7 (Z3276), F) *E. coli* (uidA), G) *Listeria monocytogenes* (hly), or H) *Legionella pneumophila* (mip) gDNA input. Inset. Reaction product from qPCR amplification of A) invA (288 bp fragment), B) groEL2 (164 bp fragment), C) nuc (279 bp fragment), D) mecA (222 bp fragment), E) Z3276 (178 bp fragment), F) uidA (500 bp fragment), G) hly (106 bp fragment), H) mip (159 bp fragment) from 1 ng gDNA input. Biotium's DNA ladder was run in lane 1. The no template control reaction is in lane 2. The PCR product is in lane 3. The 1% agarose 1X TBE gel was post-stained with 3X GelRed® in water and imaged on a UVP GelDoc-iT® using UV illumination and an ethidium bromide filter (3 second exposure).

Table 7. Troubleshooting

Problem	Solutions
No positive qPCR signals are seen in any sample above the No Template Control (NTC).	Optimize the qPCR reaction using purified genomic DNA from the same cell/strain type that you are using in your viability PCR experiment. Ensure that your cycling program works well. Increase the template volume, if necessary.
High dCt seen in the live cell control sample.	The expected dCt value for a live cell control sample is less than 1. A higher-than-expected dCt value in the live cell control sample generally indicates that the viability dye has penetrated through the live cell membrane. • Confirm that your cells are actually alive. Use a dead cell stain such as Ethidium Homodimer III (Cat. no. 40051) to measure the membrane integrity of your cells. • Ensure that there is no detergent present in your samples. • Ensure that you are not freezing your samples prior to PMA or PMAxx™ treatment. • Try a lower dye concentration: titrate the dye until you get an effective concentration that only alters dead cell DNA. • If you are treating your cells in a simple buffer (i.e., PBS) or water, try treating them in media, or buffer containing BSA or other blocking protein.
Low dCt seen in the dead cell control sample.	The expected dCt value for a heat-killed dead cell control sample is greater than 4. A lower-than-expected dCt value in the killed cell control sample may be caused by several factors. • Confirm that your cells are actually dead. Use a dead cell stain such as Ethidium Homodimer III (Cat. no. 40051) to measure the membrane integrity of your cells. • Try a higher dye concentration: titrate the dye until you get an effective concentration that only alters dead cell DNA. • Be sure to use the same volume of eluted DNA in each PCR reaction (i.e., do not normalize to ug of DNA (see "Considerations for viability PCR" #7 for additional details). • If your samples are complex (such as soil or feces), it may be helpful to try diluting them in sterile PBS, increasing the light exposure with more frequent mixing, and increasing the PMA or PMAxx™ concentration up to 200 uM.

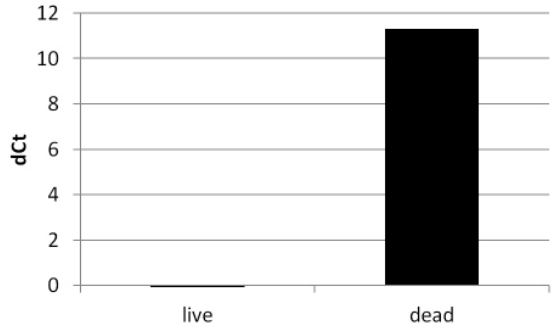


Figure 6. The dCt of live and killed E. coli with and without PMAxx™ treatment. The Ct threshold values were determined for the qPCR curves in Figure 3C. The difference in Ct (dCt) between treated and untreated samples was calculated by subtracting the Ct of the untreated sample from the Ct of the PMA or PMAxx™ treated sample.

Related Products

Catalog number	Product
E90002	PMA-Lite™ LED Photolysis Device
40013	PMA Dye, 1 mg
40019	PMA Dye, 20 mM in dH2O
40069	PMAxx™ Dye, 20 mM in dH2O
31038	PMA Enhancer for Gram Negative Bacteria, 5X Solution
31043, 31044	Forget-Me-Not™ Universal Probe qPCR Master Mix
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-color tracking)
31022	Ready-to-Use 1 kb DNA Ladder, 150 applications
31032	Ready-to-Use 100 bp DNA Ladder, 150 applications
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in water
32000-1	Live Bacterial Gram Stain Kit
32001	Bacterial Viability and Gram Stain Kit
30027	Viability/Cytotoxicity Assay Kit for Bacterial Live and Dead Cells

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.