Biotium
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Product Information

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PMA™ Real-Time PCR Bacterial Viability Kit - *Mycobacterium tuberculosis* (groEL2)

Catalog Number: 31034
Unit Size: 1 kit (200 PCR reactions)

Kit Contents

Component	Size
40019: PMA™ dye, 20 mM in H_2O	1 X 100 uL
31003A: Fast EvaGreen® qPCR Master Mix	2 X 1 mL
99939: 10X ROX Reference Dye	1 X 1 mL
31034A: groEL2 primer mix, 5 uM each primer For: 5'-CTAGGTCGGGACGGTGAGGCCAGG-3' Rev: 5'-CATTGCGAAGTGATTCCTCCGGAT-3'	1 X 400 uL

Storage and Handling

Store kit at -20 °C. Protect PMA and Fast EvaGreen Master Mix from light. Components are stable for at least 6 months when stored as recommended. Before use, thaw at room temperature and mix well by gentle vortexing. Keep Fast EvaGreen qPCR Master Mix on ice before use. Kit components are stable for several freeze/thaw cycles.

Spectral Properties

PMA: λabs = 464 nm (before photolysis);
λabs /λem = ~510/~610 nm (following photolysis and reaction with DNA/RNA)

EvaGreen: λabs = 471 nm (without DNA)
λabs/λem = 500/530 nm (with DNA)

Product Description

PMA-PCR kits are designed for selective detection of viable bacteria from a specific strain using PMA dye and real-time PCR. The kits contain PMA™ dye, Fast EvaGreen® qPCR Master Mix, and PCR primers for detection of a specific strain of bacteria. Kits are available for detection of a selection of bacterial strains that are of widespread interest to food safety, public health, and antibacterial research.

This kit contains primers for amplification of *Mycobacterium tuberculosis* groEL2 gene, with reagents sufficient to treat 80 bacterial cultures with DNA and perform 200 PCR reactions. The number of samples that can be treated with PMA using the kit may vary depending on sample type. See the product protocol under the downloads tab and references for more information.

PMA is a high affinity photoreactive DNA binding dye developed by Biotium. The dye is weakly fluorescent by itself but becomes highly fluorescent upon binding to nucleic acids. It preferentially binds to dsDNA with high affinity. Upon photolysis, the photoreactive azido group on the dye is converted to a highly reactive nitrene radical, which readily reacts with any hydrocarbon moiety at the binding site to form a stable covalent nitrogen-carbon bond, thus resulting in permanent DNA modification. The dye is cell membrane-impermeable and thus can be used to selectively modify DNA from dead cells with compromised membrane integrity, while leaving DNA from viable cells intact. PMA inhibits PCR amplification of modified DNA templates by a combination of removal of modified DNA during purification and inhibition of template amplification by DNA polymerases (1). Consequently the dye is useful in the selective detection of viable pathogenic cells by quantitative real-time PCR.

Fast EvaGreen Master Mix is a ready-to-use hot-start mix for qPCR and DNA melt curve analysis of PCR amplicons. It is formulated for qPCR using a fast cycling protocol, but also can be used for qPCR using regular cycling protocols. EvaGreen dye is a unique DNA-binding dye with features ideal for both qPCR and melt curve analysis. EvaGreen dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration in qPCR without PCR inhibition. Fast EvaGreen Master Mix contains Cheetah™ Taq, Biotium's fast-activating chemically-modified hot-start Taq polymerase, which is particularly suitable for fast PCR cycling protocols.

Mycobacterium tuberculosis is a pathogenic bacteria that infects the lungs and causes the disease tuberculosis. PCR to detect Mycobacterium tuberculosis has been reported using the primers provided in the kit (2), and these primers have been validated at Biotium for real-time qPCR using EvaGreen Master Mix (Figures 1-3). Note: groEL2 primers also amplify other mycobacteria species (2), but products may be distinguishable by melt curve analysis. An example of PMA-PCR using *E. coli* is shown in Figure 4.

Protocol for treating bacteria with PMA for qPCR

The following is a general protocol for treating cultured laboratory strains of bacteria with PMA. Treatment of complex biological or environmental samples such as feces or soil may require optimization of sample dilution for PMA-treatment, light treatment, and extraction of genomic DNA. See References 2 and 3 for more information on optimization of PMA-based viability PCR applications.

1. Inoculate an appropriate media broth with bacteria (volume is dependent on size of experiment).
2. Shake cultures at 200 RPM at 37 °C overnight.
3. Continuing culturing bacteria until the OD_{600} of the culture is approximately 1.
4. For positive control samples, heat inactivate bacteria at 100 °C for 10 min. To confirm heat inactivation of bacteria, plate 250 uL of control and heat inactivated bacteria on the appropriate media plate. Seal the plate with Parafilm and place at 37 °C. Check for colony growth at 24 hours, and again after 3-6 days.
5. Pipette 500 uL aliquots of bacterial culture into clear microcentrifuge tubes. Two tubes should be prepared for each sample to compare treatment with PMA versus no dye.
6. Briefly centrifuge the vial of PMA to collect the solution at the bottom of the vial.
7. Add the appropriate volume of PMA stock for a final concentration of 50 uM (e.g., 1.25 uL of 20 mM stock in 500 uL).
8. Incubate tubes in the dark for 5 minutes at room temperature. Flick tubes occasionally to mix, or incubate on a rocker covered with aluminum foil.
9. Expose samples to light to cross-link PMA to DNA. See information on light sources above.

    a. For light exposure using a halogen light source: lay tubes on a block of ice set 20 cm from the light source. The ice block should be in a clear tray, set on a shaker or rocking platform to ensure continuous mixing during light exposure. Set the lamp so that the light source is pointing directly downward onto the samples (up to 45 degree downward slant is OK). Place a piece of aluminum foil under the clear tray to reflect the light upward toward the bottom of the tubes. Expose samples to light for 5 min.

    b. For blue LED photoactivation systems, such as the PMA-Lite™ LED Photolysis Device, follow the manufacturer's instructions for light exposure of samples.
10. Pellet cells by centrifuging at 5,000 x g for 10 minutes. If no pellet is visible, centrifuge again at maximum speed for 5 minutes.
11. Extract genomic DNA for qPCR analysis using a standard protocol or commercially available kit. Use an appropriate protocol or kit for DNA extraction from complex biological or environmental samples (e.g., feces or soil).
12. Perform real time PCR (see following protocol). DNA templates modified with PMA will show delayed amplification by qPCR. The number of dead cells is proportional to the difference in Ct value with and without PMA treatment (see Fig. 4 for an example).

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 Fast EvaGreen Master Mix	10 uL	1X
groEL2 primer mix, 5 uM	2 uL	0.5 uM each
Template	x uL See Note 1	See Note 1
ROX	Optional	See Table 1
dH_2O	Add to 20 uL	

Note 1: Template volume should not exceed 10% of final reaction volume. Part of the proposed mechanism of action of PMA 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 volume of gDNA eluate from each sample. For live cell controls 1 ng of gDNA per reaction should be sufficient for achieving good signal. For gDNA extracted from bacterial cultures using a commercial extraction kit, 1-2 uL of eluted DNA can be used as a starting point for optimization.

Fast-cycling parameters for groEL2 real-time PCR on *M. tuberculosis* gDNA

Hold	
95 °C for 2-10 minutes (see Note 2)	
Cycling	
95 °C for 5 seconds	Cycle 40 times
55 °C for 30 seconds (acquire data)	
Melt	
57 °C to 99 °C	

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

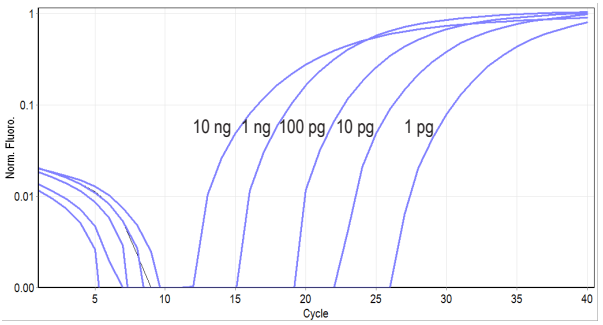

Figure 1. Fast EvaGreen® qPCR Master Mix was used to amplify a fragment of groEL2 from 10 ng, 1 ng, 100 pg, 10 pg, or 1 pg of *M. tuberculosis* gDNA (ATCC). The real-time PCR was performed on a RotorGeneQ (Qiagen).

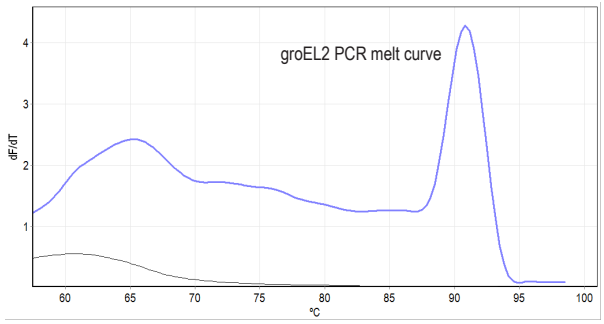

Figure 2. Melt curve analysis of the groEL2 real-time PCR product generated in Figure 1, from 1 ng of *M. tuberculosis* gDNA input.

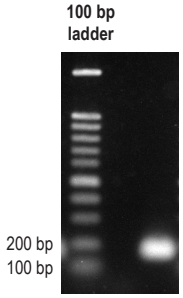

Figure 3. Reaction product from real-time PCR amplification of groEL2 (164 bp fragment) from 1 ng *M. tuberculosis* gDNA input. Biotium's 100 bp DNA ladder was run in the first lane. The 1% agarose 1X TBE gel was post-stained with 3X GelRed in water and imaged on a UVP GelDoc-iT using UV illumination and an ethidium bromide filter (3 second exposure).

Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended Rox Concentration	Amount of 10X ROX per 20 uL reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Rotor-Gene Q, Rotor-Gene3000, Rotor-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LightCycler 480, LightCycler 2.0	No ROX	None
ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P, MX3005P	Low ROX 0.05-0.1X final	Dilute 10X ROX 1:10 with dH2O to obtain 1X ROX; add 1 to 2 uL of 1X ROX per 20 uL reaction
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX 1X final	2 uL of 10X ROX per 20 uL reaction

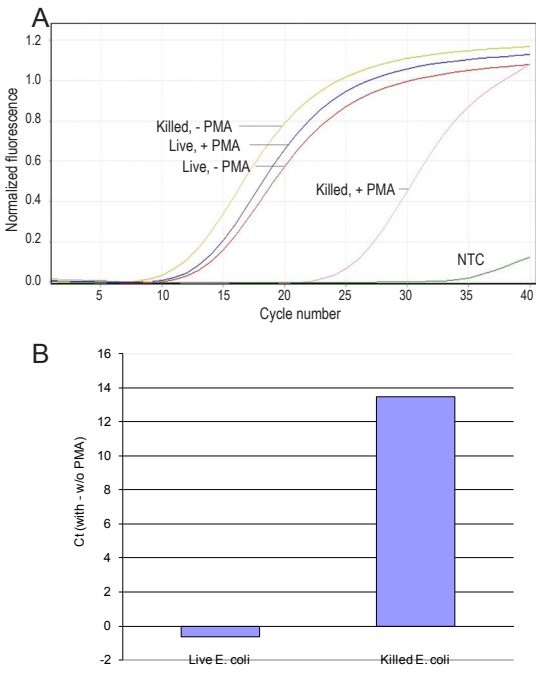

Figure 4: Effect of PMA on qPCR of DNA from live and heat-treated E. coli. qPCR was performed using primers against a region of the 16S rRNA gene. (A) Representative amplification curves for real-time PCR performed on DNA from PMA-treated live and heat-killed E. coli. (B) The Delta Ct of live and killed 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).

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

3. Fittipaldi, M., et al. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification. J. Microbiol. Meth. 91(2), 276-289 (2012).

Related Products

Catalog number	Product
E90002	PMA-Lite™ LED Photolysis Device
40013	PMA™ dye, 1 mg
31033	PMA Real-Time PCR Bacterial Viability Kit - Salmonella enterica (invA)
31035	PMA Real-Time PCR Bacterial Viability Kit - Staphylococcus aureus (nuc)
31036	PMA Real-Time PCR Bacterial Viability Kit - Staphylococcus aureus (mecA)
31037	PMA Real-Time PCR Bacterial Viability Kit - E. coli 0157:H7 (stx1)
40019	PMA™ dye, 20 mM in dH2O, 100 uL
31003-1	Fast EvaGreen® qPCR Master Mix (200 rxn), 2 x 1 mL
31021	1 kb DNA Ladder (100ng/uL), 300 ug/300 uL
31022	Ready-to-Use 1 kb DNA Ladder, 150 applications (1.5 mL)
31031	100 bp DNA Ladder, 30 ug/300 uL
31032	Ready-to-Use 100 bp DNA Ladder, 150 applications (1.5 mL)
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water, 0.5 mL
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.