PMA qPCR live bacteria detection kit

– E. coli (uidA)

Introduction

PMA qPCR live bacteria detection kit provides an effective method for detecting bacterial viability. The kit includes PMA dye and a SYBR Green-based qPCR mix. PMA is a high-affinity DNA-binding dye that primarily binds to double-stranded DNA and emits bright fluorescence upon binding. Since PMA cannot penetrate cell membranes, it only modifies the DNA of dead cells with damaged membranes. After exposure to blue light (~464 nm), the photosensitive azide groups on PMA are converted into highly reactive nitrene radicals, forming stable covalent nitrogen-carbon bonds, which result in permanent modification of the DNA, making it insoluble and lost during subsequent genomic DNA extraction. Unbound PMA decomposes into non-crosslinking hydroxylamine compounds under strong light and no longer binds to DNA. Thus, leveraging PMA's properties, the PMA-qPCR method combines PMA with qPCR technology for a novel detection approach, used for live bacterial screening. This method has been validated in various bacteria, yeasts, fungi, viruses, and parasites.

Components and Storage

Size / Components	K1544- 20tests	K1544- 200tests	Storage
PMA Dye, 20 mM in H_2O	10 µL	100 µL	-20°C, protect from light
Universal SYBR Green qPCR Super mix	0.2 mL	2 mL	-20°C, protect from light
Primer mix（uidA, 5 µM) **For: 5'-CGGTGATATCGTCCACCCAG-3'** **Rev: 5'-TGGATCGCGAAAACTGTGGA-3'**	40 µL	400 µL	-20°C, protect from light
Shipping: Blue Ice	**Shelf life:** 12 months		

Precautions

1. This live bacterial detection kit differentiates between dead and live bacteria based on cell membrane permeability. Many methods of bacterial killing can damage the cell membrane and are therefore compatible with this kit. However, some methods, such as UV irradiation, may not immediately cause cell membrane rupture. Therefore, before using this kit, you should review the literature and perform preliminary experiments to determine if the kit is suitable for the type of bacteria and killing method you are using.
2. After PMA treatment, photolysis is required to covalently bind the dye to dead cell DNA. Photolysis can be

performed using blue or white light sources. Generally, the brighter the light, the more efficient the photolysis step. Non-LED lights (such as halogen lamps) may heat your samples and negatively affect the analysis, so ice should be used to cool the samples during irradiation.

3. Samples can be stored frozen after photolysis. Freezing samples before PMA treatment and photolysis may damage the cell membrane and result in false-negative results. If freezing samples before testing is necessary, it is recommended to conduct preliminary experiments first.

4. One mechanism of PMA is to remove PMA-covalently modified DNA by precipitation from the sample. Therefore, when extracting genomic DNA, use the same volume of genomic DNA elution buffer for volume normalization. Positive controls can use genomic DNA from live cells.

5. To verify the effectiveness of PMA in the test samples, compare the Ct (dCt) values between samples treated with PMA and those without PMA.

Preparation

- Light source (for the photolysis step after PMA modification of DNA);

- Bacterial genomic DNA extraction kit;

Protocol

1. Transfer 10 μL of *E. coli* culture into liquid LB medium and incubate overnight or longer in a bacterial incubator until the bacteria reach the logarithmic growth phase (OD600 ≈ 1.0).

***Note:** The type of medium and incubation time should be adjusted according to the experiment.

2. Take two aliquots of live *E. coli*, 400 μL each, and place them into clean centrifuge tubes.
3. (Optional) Prepare dead bacteria. If dead bacteria are needed as a control, place the live bacteria into a water bath at 95°C for 5 minutes, or at 58°C for 3 hours. Choose the specific method based on the type of sample to obtain dead bacteria. Follow-up operations for dead bacteria are the same as for live bacteria.

4. For the two aliquots of live *E. coli*, treat one with 25 μM PMA and leave the other untreated.

5. Incubate the PMA-treated samples on a shaking incubator at room temperature, protected from light, for 10 minutes to allow the dye to mix thoroughly with the sample.

6. Expose the samples to light, using a blue or white light source. The exposure time should be determined experimentally. For example, use a 60 W blue light lamp and irradiate for 15 minutes.

***Note:** If using a halogen lamp, it is recommended to place the PMA-treated sample tubes on ice 20 cm away from the light source. The ice should be in a transparent tray, and adjust the light source to directly target the sample, with photolysis for 5-15 minutes.

7. Centrifuge treated and untreated live bacteria at 5000 × g for 10 minutes, then discard the supernatant.

8. Choose an appropriate genomic DNA extraction kit based on the sample type, and use the same elution volume for all samples in each group.

*Note: Refer to the instruction manual of the DNA extraction kit for the extraction steps.

9. Prepare the reaction mixture as follows:

Component	20 μL Reaction Volume	Final Concentration
Universal SYBR Green qPCR Super mix	10 μL	1×
F, R Primers	As needed	Each 0.4 μM
Template	As needed	/
H_2O	To 20 μL	

*Note: ① Extension time can be adjusted according to the instrument used; ② Taq enzyme in the Mix typically activates within 2 minutes, but genomic DNA may require a longer denaturation time. In this case, denaturation time can be extended as needed, and the specific duration should be adjusted based on the sample type.

10. Gently vortex the reaction mixture and transfer a fixed volume into PCR tubes.

11. The testing program is as follows:

Program	Temperature	Time	Cycles
Pre-denaturation	95℃	120 s	1 cycle
Denaturation	95℃	15 s	40 cycles
Annealing	60℃	30 s	
Extension	72℃	25 - 30 s	

*Note: ① For DNA extracted using commercial DNA extraction kits, start with a qPCR template volume of 2 μL for optimization; ② The template volume should not exceed 10% of the final reaction volume; ③ For gDNA as a template, a concentration of 1-10 ng is typically sufficient; ④ The final concentration of PCR primers is usually 0.4 μM for optimal results. If the reaction performance is poor, adjust the primer concentration within the range of 0.2-1 μM.

12. (Optional) Data Analysis

Use live and dead bacteria as controls to analyze and calculate the number of viable cells in the sample. It is recommended to validate primers and PCR program suitability before starting PMA qPCR live bacteria detection.

Calculation of dCt for dead and live bacteria:

(1) After qPCR, use the instrument software to calculate the Ct value for each sample.

(2) Determine if PMA successfully inhibited the amplification of dead bacteria DNA by calculating the dCt for each control bacterium as follows:

$$dCt_{live} = Ct_{(live,\ PMA\text{-}treated)} - Ct_{(live,\ non\text{-}PMA\text{-}treated)}$$

$$dCt_{dead} = Ct_{(dead,\ PMA\text{-}treated)} - Ct_{(dead,\ non\text{-}PMA\text{-}treated)}$$

(3) The dCt value for live bacteria should be close to 0±1, indicating that PMA does not affect the amplification of live cell DNA.

(4) The dCt value for dead bacteria should be greater than 4 (dCt of 4 indicates a reduction of about 16-fold, i.e., 94% of dead bacteria DNA removed; dCt of 8 indicates a reduction of about 250-fold, i.e., 99.6% of dead bacteria DNA removed).

(5) The dCt for dead bacteria depends on various factors, including strain/cell type, method of bacterial killing, PMA concentration, and amplification sequence length.

13. (Optional) Calculation of the proportion of live bacteria:

If the control results for dead and live bacteria are normal, proceed to calculate the proportion of live bacteria in the sample.

(1) Calculate the dCt value for the sample:

$$dCt_{sample} = Ct_{(sample,\ PMA\text{-}treated)} - Ct_{(sample,\ non\text{-}PMA\text{-}treated)}$$

(2) Convert the dCt value to the proportion of live bacteria:

$$\text{PMA inhibition factor} = 2^{\wedge\ (sample\ dCt)}$$

$$\text{Live bacteria \%} = 100 / \text{PMA inhibition factor}$$

14. (Optional) Calculate the absolute number of live bacteria

To calculate the absolute number of live bacteria in the sample, a standard curve should be prepared using genomic DNA from a known quantity of target bacteria. It is recommended to prepare several dilutions of genomic DNA concentrations within the range of the qPCR analysis system.

(1) Plot the Ct value on the y-axis and the cell number on the x-axis to create a standard curve.

(2) Calculate the copy number of the experimental sample:

$$Ct = \text{slope} * \text{cell number} + \text{y-intercept}\ (y = mx + b)$$

$$\text{Bacterial count}_{sample} = (Ct - \text{y-intercept}) / \text{slope}$$

***Note:** During purification, live bacterial DNA should not be lost.

Example:

E. coli (uidA)

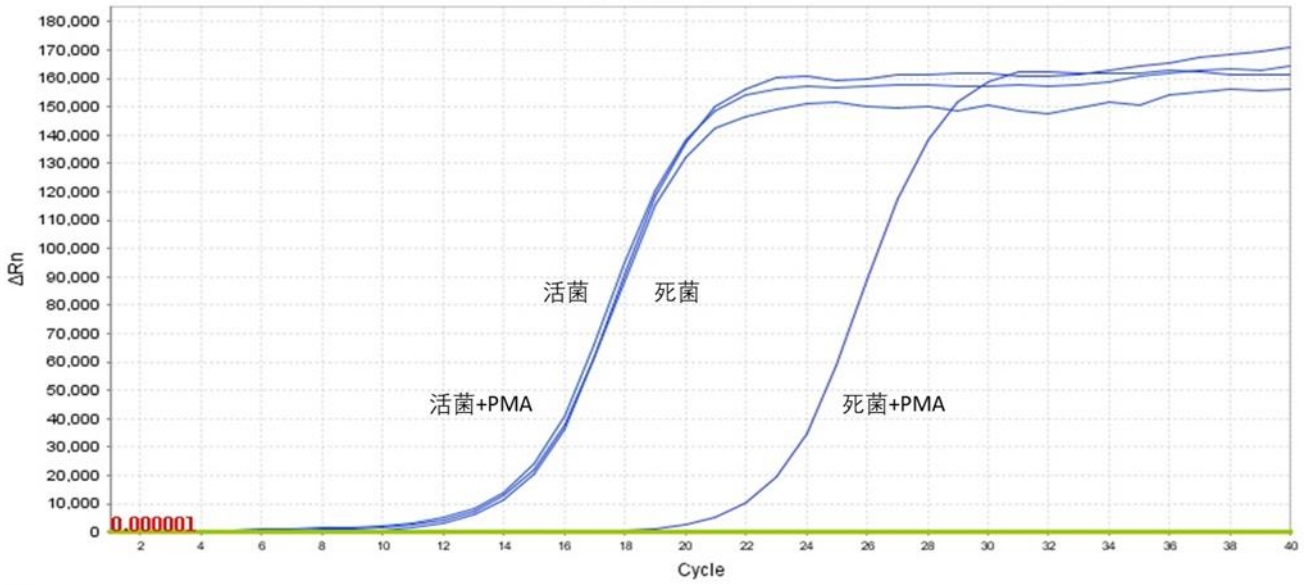

Figure 1 Live/dead *E. coli* are incubated with 25 μM PMA, followed by photolysis. DNA extraction, amplification using uidA primers

Note

1. Before use, briefly centrifuge the product to the bottom of the tube, and then proceed with the subsequent experiments.
2. The components of the kit contain fluorescent dyes; avoid light during use and storage.
3. This product is intended for research purposes only and must not be used for clinical diagnosis or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com