Product Information

Propidium Monoazide (PMA), 20mM in water

Catalog Number: P4051

Product Size: 100 µL

Parameters

Appearance: Orange red liquid

Abs = 464 nm (before photolysis)

Abs/Em: 510/610 nm (after photocrosslinking to nucleic acid)

Molecular Weight: 511

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

PMA is a high-affinity DNA-binding dye that itself has weak fluorescence but binds to nucleic acids to emit brighter fluorescence. It has a high affinity especially for double-stranded DNA. PMA does not penetrate the cell membrane and therefore can only selectively label exposed DNA on dead cells. This property allows PMA to be widely used for screening of pathogenic cells that can be cultured by means of real-time quantitative PCR (qPCR) because PMA can bind strongly to DNA on dead cells and cannot be used for amplification of PCR reactions(figure 1). This property of PMA allows it to be widely used for the screening of pathogenic bacteria that can be cultured by means of real-time quantitative PCR (Fig. 2).

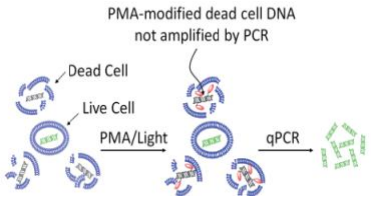

Figure 1. Principle of quantifying live and dead bacteria by qPCR after PMA modified DNA

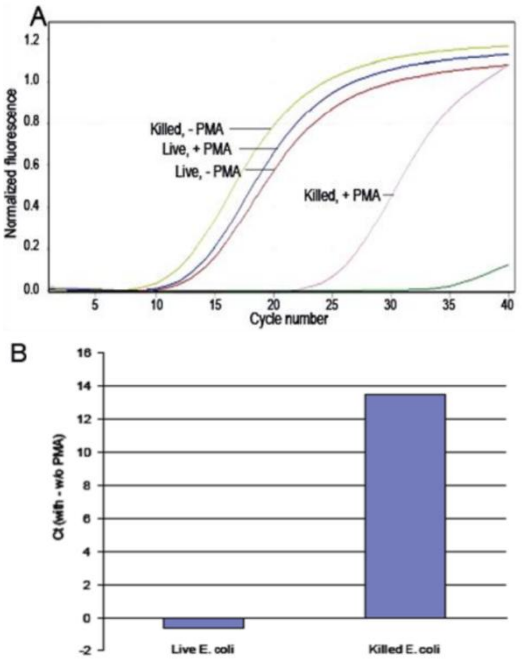

Figure 2. Using the DNA of live and heat-inactivated E. coli. as a template, after adding PMA, qPCR reaction was carried out to monitor the effect of PMA on the reaction. The primers were designed with 16S rRNA as template. (A) Amplification curve obtained by real-time quantitative PCR reaction after adding PMA; (B) PMA was added to the DNA of dead and live E. coli., qPCR reaction was performed, and the obtained Δ Ct value was compared with the negative control (Ct added to PMA - Ct without PMA).

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Protocol

1. Inoculate and amplify the bacteria with a suitable medium (the amplification volume is determined according to the specific experimental requirements);

2. 37℃, 200 RPM, overnight shaking culture;

3. Continue to culture the bacteria until the OD_{600} value of the culture suspension is close to 1;

4. Inactivate the bacteria at 58℃ for 3 h or 90℃ for 5 min to prepare a dead bacteria control sample;

5. Pipette 500 μL aliquots of the bacterial culture solution into a clean microcentrifuge tube;

6. Add appropriate amount of PMA to the microcentrifuge tube containing the bacterial suspension to a final concentration of 50 μM;

7. Incubate at room temperature for 5 min in the dark, during the incubation period, mix and mix as appropriate, or cover with aluminum foil and incubate on a shaker;

8. Expose the sample, blue or white light sources may be used. The irradiation time of different light sources should be explored to fully crosslink the PMA and DNA. For example, the sample can be illuminated with a 60W blue light for 15 min. 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;

9. Centrifuge the sample at 5,000 g for 10 min;

10. Extract genomic DNA using standard methods or kits for subsequent qPCR experiments;

11. Perform a qPCR experiment in which the PMA-modified DNA will exhibit an amplification delay effect in the qPCR reaction;

Notes

1. The conditions of the label vary depending on the cell type. Before each experiment, determine the optimal conditions. The above methods are for reference only.

2. The length of the amplified fragment is generally shorter than 100 bp. When the amplified fragment is larger than 100 bp, the signal of the heat-inactivated PMA-added cells will be weakened.

3. When preparing a positive control, usually 1 ng of live cell genomic DNA is sufficient to obtain good signal values. Therefore, when extracting genomic DNA using a commercial extraction kit, it is recommended to use 1-2 μL of DNA eluent as a starting point for optimization.

4. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

5. For your safety and health, please wear lab coats and disposable gloves

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