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PMA

Product Number: BCPMA-1mg

Description

PMA is a DNA binding dye with high affinity. The dye itself has weak fluorescence, but it can emit brighter fluorescence after binding with nucleic acids. It especially has high affinity with double stranded DNA. PMA does not have cell membrane permeability, so it can selectively modify the DNA of dead cells with damaged membranes. After bllight (~464nm) photolysis of PMA modified DNA, the photoreactive azido group on PMA is converted into highly reactive azene radical, which reacts with any hydrocarbon moiety near the DNA binding site to form a stable covalent nitrogen carbon bond, resulting in permanent DNA modification (Figure 1). This modification process will make DNA insoluble and make it lost together with cell debris in the subsequent genomic DNA extraction process, thus hindering the PCR amplification of target DNA in dead cells. The unbound PMA remaining in the solution reacts with water molecules under strong light irradiation and decomposes into hydroxylamine compounds without cross-linking activity, so that hydroxylamine can no longer covalently bind DNA, thus not affecting PCR amplification. This feature enables PMA to detect a variety of sample types including bacteria, biofilms, yeast, fungi, viruses and eukarYOtic cells by real-time quantitative PCR (qPCR); Combined with qPCR, ngs, Sanger sequencing and lamp technology, it is widely used in food and water safety and environmental testing.

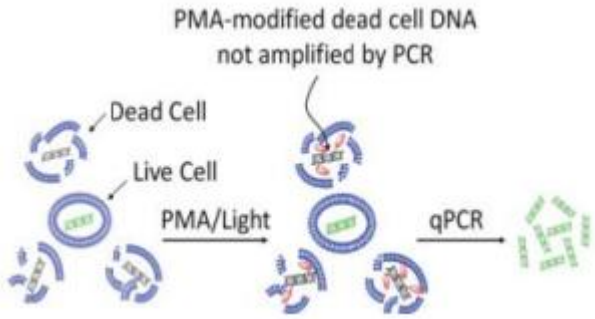

Figure . Principle of qPCR quantification of live and dead bacteria after PMA modified DNA

Application

Bacterial nucleic acid staining.

Product parameters

Product parameters: Deep red solid ; ex (pH 3) = 464nm (before photolysis) ; ex / Em (after photocrosslinking to nucleic acid) = 510 / 610nm

Usage

1. PMA distinguishes dead and live bacteria according to cell membrane permeability. Many methods of killing bacteria can cause cell membrane damage, so they are suitable for PMA detection. But some methods, such as ultraviolet irradiation, may not immediately cause cell membrane rupture. Therefore, before selecting PMA detection, it is necessary to conduct literature search and pre-experiment to determine whether it is suitable for the bacterial type and killing method you choose.
2. After PMA treatment, the bacteria need to be photolyzed to covalently bind the dye to dead cell DNA.
   Note : Photolysis operation can use blue or white light source. Generally speaking, the brighter the lamp, the higher the efficiency of the photolysis step. Non-LED lamps (such as halogen lamps) may heat your sample and have a negative impact on the analysis. Ice is required to cool the sample during irradiation.
3. Sample can be cryopreservation after photolysis. Frozen samples before PMA treatment photolysis may damage the cell membrane and produce false negative results. If the sample needs to be frozen before detection, it is recommended to perform a

pre-experiment first.

4. Part of the mechanism of PMA is to remove PMA covalently modified DNA from the sample by precipitation ; therefore, when extracting genomic DNA, it is necessary to use the same volume of genomic DNA eluent for volume normalization. The positive control can use the genomic DNA of living cells.
5. In order to verify the effectiveness of PMA in the test sample, the Ct (dCt) changes between- / + PMA can be compared.

Experimental materials (self-provided)

Light source (used for the photolysis step after PMA modified DNA) ; Bacterial genomic DNA extraction kit ; qPCR Mix kit ; effective qPCR primers corresponding to the sample type

Preparation of storage liquid before experiment： A total of 98μL of ultrapure water was added to the tube and vortexed to prepare a 20mM PMA stock solution.

Protocol

1. Suck 10μL of bacterial liquid in liquid LB medium, culture overnight or longer in the bacterial incubator, so that the bacterial culture to the logarithmic growth phase (OD600≈1.0).

   Note : The type of culture medium and culture time were adjusted according to the experiment.
2. Two copies of viable bacteria, 400μL each, were placed in a clean centrifuge tube.
3. (Recommended) Preparation of dead bacteria. If dead bacteria are needed as a control, live bacteria can be placed in a water bath at 95℃ for 5 min, or heated at 58℃ for 3 h. The specific operation is self-selected according to the sample type to obtain dead bacteria. The subsequent operation of dead bacteria is the same as that of live bacteria.
4. Two live bacteria, one without PMA treatment, and one with 25μM PMA treatment (the optimal PMA concentration for different types or different sources of bacteria needs to be referred to the relevant literature).
5. The PMA-treated samples were placed on a shaker at room temperature and incubated in the dark for 10 min to fully mix the dye with the sample.
6. Exposure of the sample, you can use blue or white light source, irradiation time to explore their own. For example, a 60 W blue light can be used for 15 min.

   Note : If a halogen lamp is used, we recommend that the PMA-treated sample tube be placed on an ice block 20cm away from the light source. The ice should be placed in a transparent tray, and the light source should be adjusted to point directly to the sample for photolysis for 5-15 min. If the bacteria obtained from the environment are directly used for experiments, due to the complexity or turbidity of the environmental samples, the photolysis time needs to be prolonged appropriately.
7. Treated and untreated live bacteria 5000×g, centrifuged for 10 min, and the supernatant was removed.
8. Select the appropriate genomic DNA extraction kit according to the sample type, and use the same elution volume for each group of samples when elution DNA.

   Note: DNA extraction steps refer to the instructions of the kit used. Part of the mechanism of action of PMA is to remove PMA-bound DNA from the sample by precipitation ; therefore, when extracting genomic DNA, each group should use the same volume of genomic DNA eluent for volume normalization (the amount of genomic DNA extracted from dead bacteria and live bacteria is inconsistent, so the concentration of the two is significantly different).
9. Preparation of reaction mixture according to the following system :

Reaction components	20μL reaction volume	Final concentration
qPCR Mix Kit (self provided)	-	1×
F. R primer	Appropriate amount	0.4μM each
Template	Appropriate amount	-
H_2O	Make up to 20μL	

Note : For the DNA extracted by commercial DNA extraction kit, the qPCR template was optimized with 2μL as the initial volume ; The template volume should not exceed 10% of the final reaction volume ; Template concentration : gDNA as template, usually 1-10ng ; the final concentration of PCR primers is usually 0.4μM, which can get better results. When the

reaction performance is poor, the primer concentration can be adjusted in the range of 0.2-1μM.

10. Slightly vortex the reaction mixture, transfer the fixed volume to the PCR tube.
11. Test procedures (according to the own qPCR Mix kit program to set the program).
12. **Data analysis**

Using live bacteria and dead bacteria as controls, the number of live cells in the sample was analyzed and calculated. It is recommended to verify the suitability of primers and PCR procedures before starting PMA qPCR detection of live bacteria.

12.1. Calculation of dead and living bacteria control dCt

12.2. After qPCR, the Ct value of each sample was calculated using the instrument software.

12.3. By calculating the dCt of each control bacteria, it was judged whether PMA successfully inhibited the amplification of dead bacterial DNA. The calculation is as follows :

12.4. dCt live = Ct (live, PMA treated) -Ct (live, PMA untreated)

12.5. dCt die = Ct (die, PMA treated) -Ct (die, PMA untreated)

12.6. The dCt expectation of living bacteria is close to 0±1, which indicates that PMA does not affect the amplification of living cell DNA.

12.7. The expected value of dCt of dead bacteria is large.

12.8. The dCt of dead bacteria depends on many factors, including : strain / cell type ; the way bacteria are killed ; the concentration of PMA used ; amplified sequence length.

13. **Calculation of the proportion of live bacteria**

If the control results of dead and live bacteria are normal, the proportion of live bacteria in the sample can be calculated.

13.1. Calculate the dCt value of the sample : dCt sample = Ct (sample, PMA treated) -Ct (sample, PMA untreated)

13.2. Conversion of dCt value to live bacteria ratio: PMA inhibition multiple = 2 (sample dCt); Viable bacteria % = 100 / PMA inhibition multiple

14. **Calculating the absolute number of viable bacteria**

If you want to calculate the absolute number of viable bacteria in the sample, you need to use a known number of target bacteria genomic DNA to make a standard curve. It is recommended that the diluted concentrations of several groups of genomes are within the range of the qPCR analysis system.

14.1. The standard curve was made with the Ct value as the ordinate and the cell number as the abscissa.

14.2. Calculate the copy number of the experimental samples : Ct = slope * cell number + y axis intercept (y =mx+b); Bacterial count sample = (Ct - y axis intercept) / slope

Note : The live bacterial DNA was not lost during the purification process.

Note

1. Please centrifuge the product to the bottom of the tube immediately before use, and then conduct subsequent experiments.
2. the components of the kit contain fluorescent dyes. Avoid light during use and storage.
3. for your safety and health, please wear experimental clothes and disposable gloves.