MycoLight™ PMA [Propidium Monoazide]

Catalog number: 24215
Unit size: 1 mg

Component	Storage	Amount (Cat No. 24215)
MycoLight™ PMA [Propidium Monoazide]	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

The common PCR assays have been routinely used for simple, sensitive, and specific pathogen identification. However, regular PCR assays cannot be used to distinguish between live and dead cells. The number of pathogens is always overestimated by the regular PCR assays. Viability PCR (vPCR) is an evolution of PCR. The simple pre-treatment of a test sample by specifically intercalating a photo-reactive reagent to the DNA of dead cells enables the selective PCR detection of DNA in live cells. The capability to selectively detect the DNA of living cells becomes very important, such as, in the fields of food safety, water quality control, infectious diseases, veterinary applications and ecological dynamics etc. Propidium monoazide (PMA) is one of the most common vPCR reagents for the selective detection of DNA of live cells. The PMA is a membrane-impermeable dye that selectively penetrates membranes of dead cells. Once inside a dead cell, PMA intercalates into the DNA and can be covalently cross-linked to it. This effect will strongly inhibit PCR amplification and leads to elimination of positive signals from dead cells. The PMA-based vPCR has been used for detecting emetic and non-emetic B. cereus and other bacterial pathogens. However, PMA is extremely light-sensitive, thus needs to be operated under the dark conditions. AAT Bioquest recently introduced MycoLight™ vPCR350. It is much less light sensitive (to the room light) than PMA, thus can be used under room light. It is a significantly improved version of PMA-like vPCR reagent. It is a novel non-fluorescent DNA modifier specifically designed for viability PCR (vPCR) targeting microorganisms such as bacteria, eukaryotes, viruses, and fungi. It represents a significant advancement over the widely used viability dye PMA (propidium monoazide), offering enhanced performance and specificity compared to PMA.

AT A GLANCE

Important Note

Before beginning the experiment, thaw MycoLight™ PMA at room temperature and briefly centrifuge to collect the dried pellet.

Protocol Summary

1. Prepare bacterial samples.
2. Add MycoLight™ PMA dye to bacterial samples.
3. Incubate samples for 20 minutes exposed to light (365 nm or 465 nm).
4. Centrifuge samples to remove any excess dye.
5. Extract genomic DNA from bacterial samples.
6. Perform PCR/qPCR using appropriate primers and master mix.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

MycoLight™ PMA Stock Solution

1. To prepare a 5 mM MycoLight™ PMA stock solution, add 300 µL of dH_2O to the vial of MycoLight™ PMA.

   Note: Any unused MycoLight™ PMA stock solution should be divided into single-use aliquots and stored at ≤ -20 ° C, protected from light. Avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

Important Note

For optimal results, it is recommended to conduct the experiment in low-light settings to reduce the impact of light on MycoLight™ PMA.

1. Inoculate the desired bacterial samples in a suitable media broth. Culture overnight until the OD600 reaches ~1.

   Note: This protocol is tailored for a 500 µL volume sample. Adjust the volume of the bacterial sample according to the experiment's scale.

2. **Optional:** To prepare dead cell controls, heat the bacterial sample (500 uL in a tube) at 90°C for 5 minutes.

3. Aliquot 500 µL of bacterial culture into individual clear microcentrifuge tubes. For each sample, prepare one tube for MycoLight™ PMA-treated cells and another for untreated cells (without MycoLight™ PMA dye added) to calculate dCt values.

4. Add 5 µL of MycoLight™ PMA stock solution to the bacterial samples.

   Note: Adding 5 µL of stock solution will result in a final dye concentration of 50 µM. This concentration can be used as a starting point and further optimization may be necessary to achieve optimal results.

5. Expose the samples to light at either 365 nm or 465 nm to cross-link the samples with MycoLight™ PMA to DNA.

   Note: For optimal activation, we recommend using AAT Bioquest's Photolyst™ X100 dual-channel LED device (Cat. No. PLX100) for consistent illumination at either 365 nm or 465 nm wavelengths.

   Note: If samples are generating heat, incubate them on ice.

6. Centrifuge samples at 5000 x g for 10 minutes. Then remove the supernatant without disturbing the cell pellet.

7. Extract genomic DNA using your preferred method or a commercially available kit suitable for the sample type.

8. Perform qPCR using primers targeting a specific genomic DNA sequence of your choice.

   Note: Perform qPCR using the same volume for all samples.

Normalization of the concentration of DNA is not required.

Data Analysis

1. After completing the qPCR, calculate the Ct (Threshold Cycle) value for each sample.

2. To determine if MycoLight™ PMA has sufficiently inhibited the amplification of DNA in dead cells, calculate the delta Ct (dCt) for each of your control cells using the formulas below:
   - dCt (Live) = Ct (Live MycoLight™ PMA treated sample) – Ct (Live untreated)
   - dCt (Dead) = Ct (Dead MycoLight™ PMA treated sample) – Ct (Dead untreated)

3. For the live cell control, the expected result should be close to 0. A greater difference between dead and live cells suggests that the MycoLight™ PMA treatment has effectively inhibited DNA in the dead cell samples.

EXAMPLE DATA ANALYSIS AND FIGURES

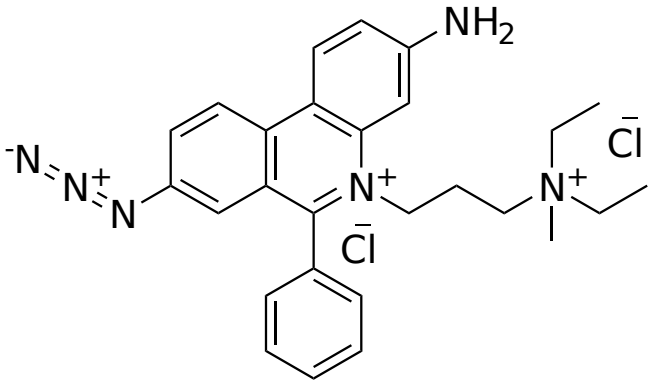

Figure 1. Propidium monoazide (PMA) is one of the most common vPCR reagents for the selective detection of DNA of live cells. The PMA is a membrane-impermeable dye that selectively penetrates membranes of dead cells. Once inside a dead cell, PMA intercalates into the DNA and can be covalently cross-linked to it.

DISCLAIMER

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