MycoLight™ vPCR350

Catalog number: 24210
Unit size: 1 mg

Component	Storage	Amount (Cat No. 24210)
MycoLight™ vPCR350	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

MycoLight™ vPCR350 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. Like PMA, MycoLight™ vPCR350 is a photo-reactive dye that strongly binds to DNA. Upon exposure to visible light, the dye covalently attaches to the DNA, effectively inhibiting its amplification by PCR. A notable feature of MycoLight™ vPCR350 is its designed impermeability to cell membranes. This unique property ensures that only dead cells with compromised membranes, are susceptible to DNA modification by the dye thereby eliminating PCR amplification of dead cell DNA and ensuring precise discrimination between live and dead bacteria. While PMA is effective at distinguishing between live and dead bacteria, it does not entirely prevent PCR products from dead cell DNA, potentially leading to false positive results. MycoLight™ vPCR350 is compatible with all existing PMA protocols. Its superior performance and reliability make it an ideal choice for microbial identification by real-time PCR (qPCR), offering a highly specific and efficient means of detection without the need to cultivate the target organisms.

AT A GLANCE

Important Note

Before beginning the experiment, thaw MycoLight™ vPCR350 at room temperature and briefly centrifuge to collect the dried pellet.

Protocol Summary

1. Prepare bacterial samples.
2. Add MycoLight™ vPCR350 dye to bacterial samples.
3. Incubate samples for 20 minutes exposed to light (Long wavelength 365 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™ vPCR350 Stock Solution

1. To prepare a 5 mM MycoLight™ vPCR350 stock solution, add 300 µL of DMSO to the vial of MycoLight™ vPCR350.

   Note: Any unused MycoLight™ vPCR350 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™ vPCR350.

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™ vPCR350 treated cells and another for untreated cells (without MycoLight™ vPCR350 dye added) to calculate dCt values.

4. Add 5 µL of MycoLight™ vPCR350 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 365 nm light using the long settings in the instrument to cross-link the samples with MycoLight™ vPCR350 to DNA.

   Note: For optimal activation, use a 3-UV lamp with wavelengths of 254/302/365.

   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™ vPCR350 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™ vPCR350 treated sample) – Ct (Live untreated)
   - dCt (Dead) = Ct (Dead MycoLight™ vPCR350 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™ vPCR350 treatment has effectively inhibited DNA in the dead cell samples.

EXAMPLE DATA ANALYSIS AND FIGURES

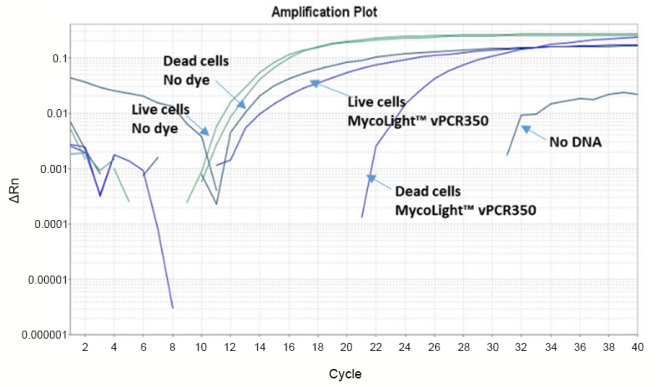

Figure 1. Normalized qPCR curves were obtained from a viability PCR experiment involving live and heat-inactivated E. coli, treated with MycoLight™ vPCR350. The qPCR analysis utilized primers targeting a region of the uidA gene. Treatment with MycoLight™ vPCR350 did not impact the amplification of DNA from live E. coli. However, it led to a notable delay in the amplification of DNA from heat-killed E. coli.

DISCLAIMER

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