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Product Information

Ethidium Monoazide Bromide (EMA)

Catalog Number	Packaging Size
C009	5 mg

Storage upon receipt:
- -20°C
- Protect from light

Ex/Em: 504/600 nm, bound to DNA

Physical Properties:

Color & Form: Orange solid
Purity: 95% by HPLC
Solubility: Soluble in DMF or EtOH

Solubility and Stock Solution Preparation

EMA is soluble in DMF or EtOH. Stock solutions can be prepared at 5 mg/mL and stable for at least 1 year at -20°C, protected from light.

Product Description

Ethidium monoazide bromide is a non-fluorescent nucleic acid stain with a photoaffinity label. The dye, after photolysis, is converted to a fluorescent DNA stain covalently bound to DNA.[1] The dye has been used to "footprint" drug binding sites on DNA[2] to modify plasmid DNA[3,4] and to determine hematopoietic cell phenotype, function and position in the cell cycle.[5] A particularly useful application of the dye is to selectively and covalently label dead cells in the presence of live cells. Because ethidium monoazide bromide is relatively impermeant to live cells, it selectively labels DNA in dead cells in a mixed population of live and dead cells. Photolysis following the dye application renders the dead cell DNA covalently labeled with the dye. One can then wash and fix the cell preparation for analysis by microscopy, fluorescence plate reader or flow cytometry. The major advantage of this method is that researchers can avoid extensive manipulation of live pathogenic organisms.[6] Ethidium monoazide bromide also has been used to differentiate between viable and dead bacteria by 5'-nuclease PCR. [7]

References

1) J. Mol. Biol. 92, 319 (1975); 2) Euro. J. Biochem. 182, 437 (1989); 3) J. Biol.Chem. 257, 13205 (1982); 4) J. Biol. Chem. 259, 11090 (1984); 5) Cytometry 11,610 (1990); 6) Cytometry, 12, 133 (1991); 7) Biotechniques, 34 (4), 804 (2003).

Protocol for treating bacteria with EMA for qPCR

The following is a protocol for treating cultured laboratory strains of bacteria with EMA. Treatment of complex biological or environmental samples such as feces or soil may require optimization of sample dilution for EMA and light treatment.

1. Inoculate an appropriate media broth with bacteria (volume is dependent on size of experiment).
2. Shake cultures at 200 RPM at 37°C overnight.
3. Continuing culturing bacteria until the OD600 of the culture is approximately.
4. For positive control samples, heat inactivate bacteria at 100°C for 10 min. To confirm heat inactivation of bacteria, plate 250 uL of control and heat inactivated bacteria on the appropriate media plate. Seal the plate with Parafilm and place at 37°C. Check for colony growth at 24 hours, and again after 3-6 days.
5. Pipette 500 μL aliquots of bacterial culture into clear microcentrifuge tubes.
6. Briefly centrifuge the vial of EMA to collect the solution at the bottom of the vial.
7. Add the appropriate volume of EMA stock for a final concentration of 50 μM (e.g., 1.25 μL of 20 mM stock in 500 μL).
8. Incubate tubes in the dark for 5 minutes at room temperature. Flick tubes occasionally to mix, or incubate on a rocker covered with aluminum foil.
9. Expose samples to light to cross-link EMA to DNA.
10. Pellet cells by centrifuging at 5,000 x g for 10 minutes. If no pellet is visible, centrifuge again at maximum speed for 5 minutes.
11. Extract genomic DNA for qPCR analysis using a standard protocol or commercially available kit. Use an appropriate protocol or kit for DNA extraction from complex biological or environmental samples (e.g., feces or soil).
12. Perform qPCR using primers against an appropriate genomic DNA target for your organism of interest. DNA templates modified with EMA will show delayed amplification by qPCR. The number of dead cells is proportional to the difference in Ct value with and without EMA treatment.