FluoProbes®

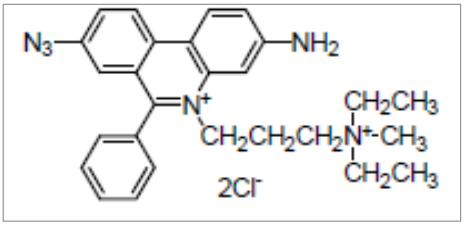
FT-BZ9340

EMA, PMA™

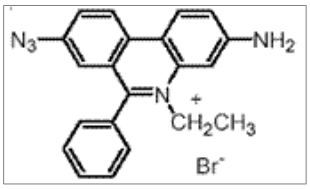
Affinity and photoreactive probes for DNA useful for dead cell staining

Products Description

Name :	**PMA™**, Propidium Monoazide Phenanthridium, 3-amino-8-azido-5-[3-(diethylmethylammonio)propyl]-6-phyenol dichloride
Catalog Number :	FP-BZ9340, 1 mg FP-FO5631, 100 µl (20mM in H_2O)
Structure :	$C_{27}H_{33}Cl_2N_6$
Molecular Weight :	MW= 512.51
Solubility:	Water, DMSO or DMF at least 10mg/ml
Absorption / Emission :	λ_{abs} (before photolysis) = 464 nm $\lambda_{exc.}/\lambda_{em}$(following photolysis and covalent attachment to DNA/RNA)= 510/610nm



Name :	**EMA**, Ethidium Monoazide
Catalog Number :	FP-48256A, 5 mg
Structure :	$C_{21}H_{18}BrN_5$
Molecular Weight :	MW= 420.32 ; CAS: [58880-05-0]
Solubility :	DMSO, DMF, Methanol, and water at least 5mg/ml
Absorption / Emission :	$\lambda_{exc.}$ (pH3) = 458 nm $\lambda_{exc.}/\lambda_{em}$ (free in water) = 462/625nm (weak) EC: 5 400 M^{-1} cm^{-1} $\lambda_{exc.}/\lambda_{em}$ (hydrolysed, DNA bound) = 504/600nm



Also available as 20mM solution in H_2O #FO5630, 100µl

Storage: Solid can be stored at +4°C or -20°C (M) Protect from light at all time and moisture
When stored as recommended, the solid dye is stable for at least one year from date of receipt.
To prepare a 20 mM stock solution, dissolve 1 mg PMA in 98 uL sterile dH_2O.

20 mM stock solution can be stored at least 6 months at -20°C protected from light. When stored as recommended the dye solution is stable for at least six months from date of receipt.

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Technical Information - EMA

Ethidium monoazide bromide is a fluorescent nucleic acid stain with a photoaffinity label. The dye, after photolysis, binds covalently to nucleic acids.[1] The dye has been used to "footprint" drug binding sites on DNA[2] to modify plasmid DNA,[3,4] and to determine hemopoietic 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. Since 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 and exam it 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]

References - EMA:

1) J. Mol. Biol. 92, 319(1975)
2) Euro. J. Biochem. 182, 437(1989)
3) J. Biol. Chem. 257, 13205(1982)
[4] Ethidium binding sites on plasmid DNA determined by photoaffinity labeling, Hardwick JM, *et al*., J Biol Chem (1984) 259:11090-11097, ID: PN6289
5) Cytometry 11, 610(1990)
6) Cytometry, 12, 133(1991)
7) Nucleic Acids Res.i 5, 4891(1978) [excitation/emission of the dye before and after binding to DNA/RNA and photolysis]

8) Gene inactivation by multiphoton-targeted photochemistry. M W Berns et al., PNAS Article 2000-08-15 - PMID 10944219 ;

[*] **Sart S.** *et al.*, Extracellular matrices decellularized from embryonic stem cells maintained their structure and signaling specificity. *Tissue Engineering, Part A: Tissue Engineering* 2014-01-01 - PMID 23848515

[*] **Käbisch R.** *et al*., Involvement of Toll-like receptors on Helicobacter pylori-induced immunity. *PLoS ONE* 2014-01-01 - PMID 25153703

[*] **Dannelley JM**, *et al.,*Efficiency of photoaffinity labeling DNA homopolymers and copolymers with ethidium monoazide. *Photochem Photobiol* (1986) 43:7-11, ID: PN6290

[*] **McMurray CT** *et al .,* Binding of ethidium to the nucleosome core particle. 2. Internal and external binding modes. *Biochemistry* (1991) 30:5644-5652, ID: PN11931

[*] **Knut Rudi** *et al.,* Use of Ethidium Monoazide and PCR in Combination for Quantification of Viable and Dead Cells in Complex Samples - *Appl. Environ. Microbiol. February* 2005 vol. 71 no. 2 1018-1024 - Article

[*] **Minami J.** *et al.,* New approach to use ethidium bromide monoazide as an analytical tool. *J Appl Microbiol.* 2010 Sep;109(3):900-9. PMID 20374413

Technical Information - PMA

PMA™ is a high affinity photoreactive DNA binding dye. The dye is weakly fluorescent by itself but becomes more fluorescent after binding to nucleic acids. It preferentially binds to dsDNA with high affinity. Upon photolysis, the photoreactive azido group on the dye is converted to a highly reactive nitrene radical, which readily reacts with any hydrocarbon moiety at the binding site to form a stable covalent nitrogen-carbon bond, thus resulting in permanent DNA modification. The dye is nearly completely cell membrane-impermeable, and thus can be selectively used to modify only exposed DNA from dead cells while leaving DNA from viable cells intact. This feature makes the dye highly useful in the selective detection of viable pathogenic cells by quantitative real-time PCR in the presence dead cells whose DNA has been PMA-modified and thus can not be amplified (Nocker 2006) .

PMA has been used for the enumeration of *Listeria monocytogenes* in the presence of **dead** cells. PMA had no antimicrobial effect on *L. monocytogenes*. Viable cell counts were linearly related to real-time PCR Ct values for PMA treated cells from planktonic and biofilm sources over a 4 log range (Pan, 2007).

PMA can be dissolved in de-ionized H_2O at 20 mM (or in DMSO or DMF at least 10mg/ml). The prepared stock solution should be stable for at least 6 months if stored at –20°C.

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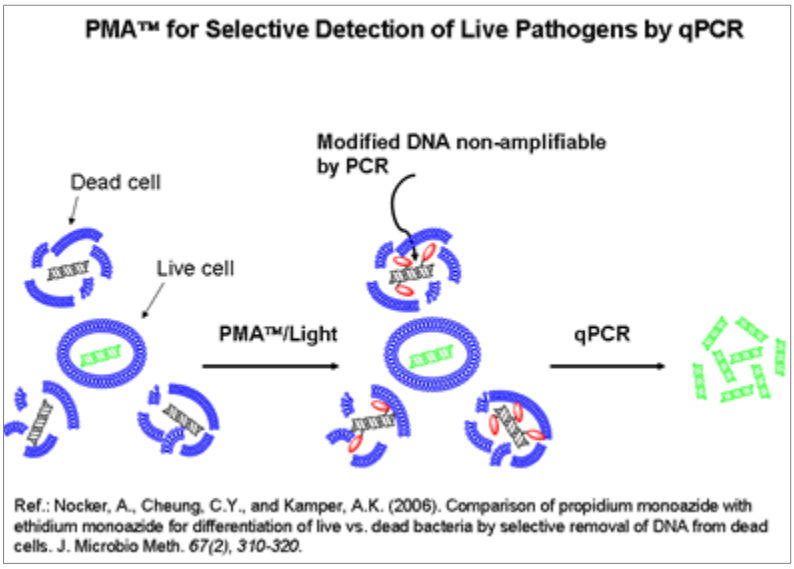

PMA treatment for DNA (Wahman, 2009)

- Dissolve PMA in 20% dimethyl sulfoxide, creating a 20 mM PMA stock solution
- Store this stock solution at –20°C in the dark
- Add PMA to culture aliquots (1.75 ml) to a final PMA concentration of 50 µM in 2-ml microcentrifuge tubes
- Vortex briefly.
- Incubate these samples in the dark for 5 min before being exposed to light for 2 min at a distance of 20 cm from a 650-W halogen light source
- To avoid excessive heating, laid the samples horizontally on ice and rotate every 30 s.
- After PMA treatment, harvest the cells by centrifugation at 5,000 x g for 10 min prior to DNA isolation.

Propidium monoazide treatment for RNA (Parshionika, 2010)

- Reconstitute PMA with 20% dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/ml
- Stored at –20°C.
- In a dark room, add 25 µl of PMA to 100 µl of sample in a 1.5-ml microcentrifuge tube
- Adjust the final concentration to 100 or 200 µM with molecular-grade water.
- Place sample tubes on a rocker, and mixe the contents for 5 min.
- After mixing, place the tubes on their sides on ice to prevent overheating and expose to a 650-W light at a distance of 20 cm for 3 min.
- Extract the RNA
- Use the extracted RNA as a template in RT-PCR and quantitative RT-PCR experiments.

If you use a halogen lamp (>600 W) for home, we recommend that you lay tubes on a block of ice set 20 cm from the light source, on a rocking platform to ensure continuous mixing. The ice block should be in a clear tray with a piece of aluminum foil under the clear tray to reflect the light upward. Set the lamp so that the light source is pointing directly downward onto the samples (up to 45° downward slant is OK). Expose samples to light for 5 min.

References - PMA

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- **Hein I**. *et al*., Possible Errors in the Interpretation of Ethidium Bromide and PicoGreen DNA Staining Results from Ethidium Monoazide-Treated DNA, *Appl. Envir. Microbiol*., 72: 6860 – 6862 (2006) Article
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- **Wahman D**. *et al.*, Monochloramine Disinfection Kinetics of *Nitrosomonas europaea* by Propidium Monoazide Quantitative PCR and Live/Dead BacLight Methods, *Applied and Environmental Microbiology,* p. 5555-5562, Vol. 75, No. 17 (2009) Article; PMDI PMC 2737938/

Related products

- Live/Dead bacterial viability kit, FP-BU1040
- GelRed, BY1740
- EvaGreen, dsDNA reagent, BI1790
- Fast EvaGreen master mix for QPCR and HRM, DV7220

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

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 Rev.P11E-I07E-

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