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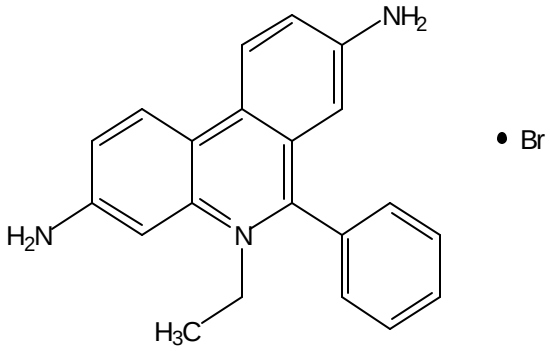
ProductInformation

ETHIDIUM BROMIDE
Product Number **E1510, E1385, E7637, and E8751**
Storage Temperature RT

Synonyms: 3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide; 2,7-Diamino-10-ethyl-9-phenylphenanthridinium bromide; Homidium Bromide; Dromilac; EtBr

Product Description



Appearance: Red to purple powder
Molecular formula: $C_{21}H_{20}N_3Br$
Molecular weight: 394.3
Melting point: 255-266°C with decomposition[1,2]

Spectral data:
UV/Vis Absorbance:
Maxima at 210 nm, E^{mM} = 0.20-0.5; 285 nm, E^{mM} = 5.0-10.0; 316 nm, E^{mM} = 50.0; 343 nm, E^{mM} = 40.0 (water)[3]
Published absorption spectrum (methanol) with λ_{max} at 296 nm and 525 nm[1]
λ_{max} = 475 nm, E^{mM} = 5.76 (0.66 M glycine buffer)[4]

Solvent effects on absorbance:
λ_{max} = 480 nm (water); 515 (glycerol); 520 nm (methanol): 520 nm (acetone); 532 nm (ethanol); 535 (DMSO) and 540 nm (pyridine).[5] The absorbance maximum relative to water is red-shifted to longer wavelengths upon binding to nucleic acids.[6,7]

Fluorescence:
Excitation / emission wavelengths reported for the EtBr-nucleic acid complex:
Ex at 526 nm, Em at 605 nm (aqueous)[8]
Ex at 360 nm, Em at 590 nm (in PBS)[9]
Ex at 525 nm, Em at 600 nm (10 mM TBE, pH 8.0)[10]
Ex (absorption) at 510 nm, Em at 590 nm.[11]
Ex (absorption) at 482 nm (blue-green), Em at 616 nm (red-orange).[12] The fluorescence yield of EtBr increases as solvent polarity decreases.[13]

Ethidium bromide is a well-known and widely used fluorescent dye in biotechnology research. Early usage was as a veterinary trypanocide.[14] It is a mutagenic compound which intercalates double-stranded DNA and RNA.[1,10] The fluorescence of EtBr increases 21-fold upon binding to double-stranded RNA, 25-fold on binding double-stranded DNA (although histones block binding of EtBr to DNA). Ethidium bromide has been used in a number of fluorimetric assays for nucleic acids.[15,16,17] It has been shown to bind to single-stranded DNA (although not as strongly)[2] and triple-stranded DNA.[18] Because of the binding to DNA, EtBr is a powerful inhibitor of DNA polymerase.[4]

E7637, Molecular Biology grade powder, is suitable for use in gel electrophoresis and DNA isolation procedures. E-1510, Aqueous Solution (10 mg per ml), is suitable for use in gel electrophoresis and DNA isolation procedures. E1385, Molecular Biology grade Aqueous Solution (500 µg per ml), is suitable for use in gel electrophoresis.

Related Products

Methylene blue (M4159) was reported as an alternative stain.[19] Thiazole orange homodimer (monomer = Aldrich Product Number 39,006-2) was noted as nonmutagenic, but useful for detecting DNA in agarose gels.[20]

For use in cell studies, dihydroethidium (Sigma Prod. No. D7008), also known as hydroethidine, is a possible alternative. It is the reduced form of the dye and is converted (dehydrogenated) inside the cell to the ethidium cation, which then intercalates into DNA.[21,22]

At present, the recommended ultimate disposal method is by incineration as discussed in the MSDS.[23] Processing solutions through Rohm and Haas Amberlite XAD-16 resin was shown to be effective to the limit of detection, with none of the completely decontaminated solution found to be mutagenic.[24, 25] Sigma offers XAD-16 as a bulk product, as well as several products that adsorb the dye for easier disposal. See Rezorian A161 cartridges (5-7611 or 5-7610) and the Extractor ™ for EtBr decontamination (Z36,156-9). A 5 ml Rezorian A161 cartridge can be used to treat more than 16 liters of solution (0.5 mg/liter of EtBr) before dye breakthrough. The luer lock cartridge can be used with 4 mm I.D. tubing, syringes or low-pressure chromatographic systems. The Extractor ™ for EtBr decontamination can process up to 10 liters of solution (0.5 mg/liter of EtBr). Other methods of treatment for disposal of aqueous EtBr solutions have been suggested.[26,27,28] Sigma has not verified and does not endorse these procedures.

Preparation Instructions

At room temperature, EtBr dissolves in water at 10 mg/ml to give a red solution.[2] It should be soluble up to 20 mg/ml in water or up to 2 mg/ml in ethanol.[1] It is soluble 1 part in 750 parts chloroform.[3]

Stock solutions of EtBr in water or PBS are stable for at least two years at room temperature if protected from light.[11]

Storage/Stability

The solid powder has shown minimal change after two years stored at room temperature, protected from light.

Procedure

Use in Electrophoresis Staining [6]

1. The dye is usually incorporated into the gel and the electrophoresis buffer at 0.5 µg/ml. Note: Electrophoresis mobility of linear double-stranded DNA is reduced by approximately 15% in the presence of the dye.
2. To stain after gel has been run, immerse gel in electrophoresis buffer or water containing EtBr (0.5 µg/ml) for 30-45 minutes at room temperature.
3. Destaining is optional. Detection of very small amounts (<10 ng) of DNA is made easier if the background fluorescence caused by unbound EtBr is reduced by soaking the stained gel in water or 1 mM $MgSO_4$ for 20 minutes at room temperature.

References

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