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6-Methoxy-N-ethylquinolinium Iodide (M-6886)

Quick Facts

Storage upon receipt:
- Room temperature
- Desiccate

Molecular Weight: 315

Ex/Em: 344/440 nm

Introduction

Several quinoline derivatives have been exploited as intracellular chloride indicators based on collisional quenching of their fluorescence by halide ions.[1] Significant limitations of existing indicators available from Molecular Probes, such as SPQ and MQAE, are that they require invasive loading procedures and exhibit rapid leakage from within loaded cells.[2-4]

To address these problems, a cell-permeant "masked" chloride indicator, 6-methoxy-*N*-ethyl-1,2-dihydroquinoline (DiH-MEQ) has been developed by Biwersi and Verkman.[5] Nonpolar DiH-MEQ is converted to the positively charged, chloride-sensitive MEQ (6-methoxy-*N*-ethylquinolinium) by intracellular oxidation. Once loaded, MEQ appears to exhibit similar chloride response characteristics to SPQ.[5] Because diH-MEQ is susceptible to spontaneous oxidation, Molecular Probes offers this novel material in its oxidized, cell-impermeant form (MEQ) together with a protocol for its reduction to DiH-MEQ.

Materials

Contents and Storage

Solid MEQ (supplied as the iodide salt) is stable for at least one year when stored desiccated at room temperature.

Materials Required but Not Provided

- Sodium borohydride ($NaBH_4$; Sigma Chemical Co., Catalog number S-9125). 200 mg of $NaBH_4$ will be more than sufficient to reduce 100 mg of MEQ.
- Anhydrous sodium sulfate or magnesium sulfate
- Suitable organic solvent: ethyl ether, ethyl acetate or chloroform

Preparation of DiH-MEQ

This protocol describes the preparation of DiH-MEQ from 5 mg of MEQ. The yield of DiH-MEQ is approximately quantitative.

1.1 Dissolve 5 mg (16 µmol; molecular weight = 315) of MEQ in 0.1 mL of distilled water in a **glass** test tube with at least 3 mL capacity. The requirement for a glass tube is dictated by the use of organic solvents that may dissolve plastic or leach organic plasticizers. Protect the solution from light (e.g. with aluminum foil).

1.2 Prepare a small amount (20–50 µL) of a 12% (120 mg/mL) aqueous solution of sodium borohydride. Sodium borohydride is unstable in water so this solution must be used immediately.

1.3 Flush the solution of MEQ with a slow stream of nitrogen or argon for 1 minute.

1.4 Slowly add 10 µL (32 µmol) of the sodium borohydride solution to the 0.1 mL of MEQ solution with continual purging with nitrogen or argon gas. The solution should first turn red then yellow. DiH-MEQ will typically separate from the solution as a yellow oil.

1.5 The reaction should be complete in about 30 minutes. This can be confirmed by spectrophotometrically monitoring the disappearance of MEQ in the supernatant.

1.6 DiH-MEQ is extracted from the reaction mixture with ethyl ether, ethyl acetate or chloroform. Use appropriate precautions to avoid exposure to solvent fumes. DiH-MEQ will pass into the organic layer, which in the case of ethyl ether or ethyl acetate will be the upper layer, and in the case of chloroform will be the lower layer. Dilute the reaction mixture with 0.5 mL water and 0.5 mL solvent. Vortex thoroughly for 30 seconds. Allow the layers to separate and pipet off the organic layer into a clean test tube. Avoid transferring any of the aqueous layer. Repeat the extraction, using another 0.5 mL solvent. Combine the organic extracts.

1.7 Dry the organic layers over 100 mg anhydrous sodium sulfate or magnesium sulfate for 5 minutes. Mix the organic layers thoroughly with the drying agent.

1.8 Transfer the organic layer to a glass storage container that can be tightly sealed.

1.9 Evaporate the organic extracts under argon or nitrogen gas *placed above the solvent* or by careful evaporation under a vacuum. Slight warming may accelerate evaporation. It is best to remove the last traces of solvent under a vacuum.

1.10 Solid DiH-MEQ should be stored in the dark, tightly sealed under nitrogen or argon. With these precautions, it appears to be stable for at least two weeks at -70°C and for at least one week at -20°C, as judged by spectrophotometric analysis. However, use of fresh preparations of diH-MEQ for each assay is strongly recommended.

1.11 Excess aqueous sodium borohydride can be destroyed before disposal by slow addition of acetone.

Application

The following general protocol for mammalian cell loading with diH-MEQ is based on the methods developed by Biwersi and Verkman.[5] More recently, this procedure has been adapted for loading live brain slices.[6]

2.1 Prepare a 25–50 µM loading solution of DiH-MEQ by dissolving the yellow oil directly in physiological buffer. If it is not directly soluble in the buffer of choice, prepare a concentrated stock solution in dimethylsulfoxide (DMSO) (25–50 mM) and then dilute an aliquot of the DMSO stock solution into the buffer.

2.2 Incubate cells with DiH-MEQ loading solution for 5 to 10 minutes.

2.3 Wash twice in physiological buffer and incubate cells at 37°C for 15 minutes to allow the dye to disperse evenly.

2.4 Visualize fluorescence with filters appropriate for the excitation and emission maxima of MEQ (344 nm and 440 nm, respectively [5]). Omega® filter set XF03 and Chroma filter set 31025 are recommended for this purpose. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).

Warning

Sodium borohydride (not supplied) releases hydrogen gas (flammable) if acidified. Dispose of solutions containing small quantities of sodium borohydride by treatment with acetone for 30 minutes.

References

1. J Heterocyclic Chem 19, 841 (1982); **2.** Anal Biochem 178, 355 (1989); **3.** Biophys J 53, 955 (1988); **4.** Am J Physiol 259, C375 (1990); **5.** Biochemistry 30, 7879 (1991); **6.** Methods 18, 197 (1999).

Product List

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Cat #	Product Name	Unit Size
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