Revised: 18–March–2001

1-(4,5-Dimethoxy-2-nitrophenyl) diazoethane Generation Kit (D-2516)

Quick Facts

Storage upon receipt:

DMNPE, Component A

- –20°C
- Desiccate
- Protect from light

Note: Components B and C may be stored at room temperature.

Introduction

Flash photolysis of photoactivatable or "caged" probes provides a means of controlling the release — both spatially and temporally — of biologically active products or other reagents of interest.[1,2] These probes have tremendous potential for use in single living cells, or with specialized equipment, in subcellular organelles. Furthermore, the chemical caging process may also confer membrane permeability on the caged ligand, as is the case for Molecular Probes' caged cyclic AMP and caged neurotransmitters. Most of the caged reagents described in the literature have been derivatives of *o*-nitrobenzylic compounds, which are activated by photolysis at <360 nm. A preferred caging group is currently 1-(4,5-dimethoxy-2-nitrophenyl)ethyl (DMNPE). Since the diazoethane precursor to DMNPE esters is unstable, Molecular Probes has assembled a kit that includes manganese (IV) oxide (MnO_2) for oxidation and the hydrazone precursor, along with tested procedures for generation of the reactive diazoethane and for coupling the diazo product to weak oxo-acids (pK_a = 3 to 7) such as carboxylates (Figure 1) or phosphates.[3] If the target molecule has multiple reactive centers, this synthetic method gives a mixture of products that require chromatographic separation.

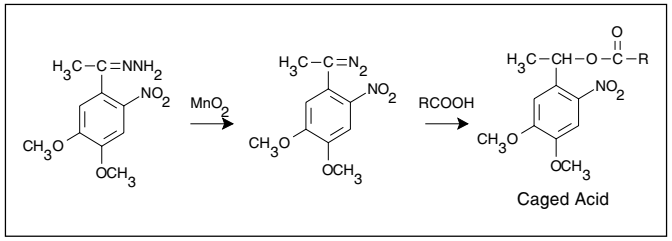

Figure 1*. The two-step reaction required for synthesizing a caged probe from the hydrazone precursor of DMNPE.*

Materials

Contents and Storage

- **4,5-Dimethoxy-2-nitroacetophenone hydrazone** (Component A), 25 mg
- **Manganese (IV) oxide** (Component B), >100 mg
- **Celite® diatomaceous earth** (Component C), >100 mg

The hydrazone should be stored desiccated, at -20°C, protected from light. Manganese (IV) oxide and Celite may be stored at room temperature.

Materials Required but Not Provided

- Chloroform, dimethylformamide (DMF) or dimethylsulfoxide (DMSO)

Experimental Protocols

Although our experience has indicated that it is not necessary to rigorously protect these reactions and the reaction products from normal ambient light, we advise that these materials should not be exposed to direct sunlight or other strong sources of UV radiation. Wrapping reaction vessels and chromatography columns with aluminum foil is a simple and worthwhile precaution. These materials appear to be less stable in solution and on silica gel than in solid form.

Preparation of Reactive Diazoethane

The diazoethane is prepared in an organic solvent, the selection of which is dependent on the solubility characteristics of the target molecule. For molecules that have high solubility in typical organic solvents, chloroform is the solvent of choice because of its volatility, which allows it to be readily evaporated from the reaction mixture. For target molecules that are water soluble but poorly soluble in most organic solvents, the diazoethane can be generated in DMF or DMSO. The half-life ($t_{1/2}$) for stability of the diazoethane is about 2 days at 21°C in chloroform.[4] Therefore, we suggest preparing the reagent just prior to use for coupling.

1.1 Dissolve 4,5-dimethoxy-2-nitroacetophenone hydrazone (25 mg, 0.10 mmol) in 1.0 mL of chloroform (or DMF or DMSO).

1.2 Add manganese (IV) oxide (100 mg, 1.15 mmol) in one portion. Allow the mixture to stir for approximately 30 minutes at ambient temperature.

1.3 Pressure filter the reaction mixture through a Celite filter pad (approximately 100 mg of Celite) contained in a small pipet, to yield a red solution. Repeatedly wash the filter pad with portions of solvent (250 µL) until the wash is near colorless.

1.4 The combined diazoethane-containing filtrate can be used for the subsequent coupling reaction without further purification. The yield of diazoethane is usually 90–95%.

Coupling to Target Molecule

2.1 The molar ratio of diazoethane reagent to target molecule is variable depending on the efficiency of the particular reaction. We have observed that, in aqueous reaction mixtures, a reaction initially occurs which consumes the diazoethane rapidly. However, this undetermined side reaction subsides and the reaction with the target molecule then proceeds. In commercial preparations of caged nucleotide triphosphates, we typically use 8 molar equivalents of the diazoethane in order to obtain a reasonable degree of reaction.

2.2 The red diazoethane solution (see step 1.4) can be added directly to a solution of the target molecule in a dropwise manner at ambient temperature. The solvent for the target molecule should be miscible with the diazoethane solvent so that a homogeneous reaction mixture is obtained. This will enhance the efficiency of the coupling. Addition of the diazoethane should result in an immediate loss of the red color and release nitrogen gas. As the target molecule is consumed, the red color should persist for increasing lengths of time between additions.

2.3 Progress of the coupling reaction may be monitored by thin layer chromatographic analysis (TLC) on silica gel plates. Decomposition of the caged target molecule may occur during the spotting process, which would result in a false reading after plate development. **DO NOT** check the quality of the spotting, prior to development, with a UV lamp.

2.4 Product mixtures can be separated chromatographically. Depending on the nature of the target molecule, we have employed silica gel, lipophilic Sephadex® or ion exchange resins. Many purified "caged" compounds can be qualitatively tested on a silica gel plate. The pure "caged" compound is spotted, the spot is exposed to UV radiation from a hand lamp for 15 minutes and the plate is developed. Visualization should show the uncaged target compound and the nitroso by-product.

Warning

Diazoalkanes are potentially explosive. Although the quantities of these materials produced by this kit are limited, all operations should be carried out in an adequately ventilated fume hood, behind a glass shield. Manganese (IV) oxide is a hazardous oxidant. Observe precautions regarding sensitivity of supplied materials and reaction products to ultraviolet light (see *Experimental Protocols*).

References

1. *Methods in Enzymology, Vol. 291*, G. Marriott, Ed., Academic Press (1998); **2.** *Optical Microscopy: Emerging Methods and Applications*, Herman B, Lemasters JJ, Eds. pp. 27–85 (1993); **3.** J Biol Chem 270, 29656 (1995); **4.** J Am Chem Soc 110, 7170 (1989).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
D-2516	1-(4,5-Dimethoxy-2-nitrophenyl) diazoethane Generation Kit	1 kit

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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