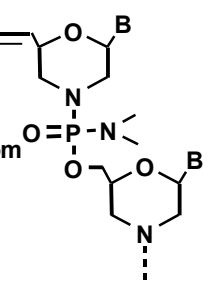
Using Morpholinos & Vivo-Morpholinos

5 Apr 2016

GENE TOOLS, LLC

Phone: (541) 929-7840
Fax: (541) 929-7841
Web: www.gene-tools.com
Email: custsupport@gene-tools.com
Mail: 1001 Summerton Way
Philomath, OR 97370 USA



CONTENTS

USE

Oligo state on shipment

Standard Morpholino oligos, Vivo-Morpholinos and Fluorescent Vivo-Morpholinos are shipped as sterile lyophilized solids.

Making a Stock Solution of a Morpholino

A Morpholino is delivered as a prequantitated, sterile, salt-free, lyophilized solid in a glass vial. We recommend making a Ò1 mM stock solution in distilled water. Due to their lower solubilities, Vivo-Morpholinos should be dissolved at lower concentration (Ò0.5 mM). Water can be removed by lyophilization if needed. It is easiest to analyze Morpholinos by MALDI-TOF from a water solution. You can make a stock solution in a buffer (e.g. Danieau buffer or Ringerôs solution); however, some salts decrease solubility of Vivo-Morpholinos and Fluorescent Vivo-Morpholinos and lyophilization or mass spectrometry will be more difficult. Like other oligos, Morpholinos can be damaged by diethyl pyrocarbonate (DEPC).

GENE TOOLS suggests storing Morpholino stock solutions at room temperature in the original GENE TOOLS vial. It is best to keep stocks at 1 mM or less to avoid insolubility or aggregation and in tightly sealed vials to prevent evaporation, ideally in a humid environment. Activity lost due to solubility issues can often be restored by autoclaving Morpholino solutions. If your oligo does not readily dissolve during stock preparation, heat the vial to 65°C and vortex. If dissolution is incomplete, autoclave the stock solution.

Stock solutions of Morpholino oligos		
Amount of Morpholino (Amount of Vivo-Morpholino)	Volume of sterile water	Resulting stock concentration
100 nanomoles	0.10 mL	1.0 milliMolar (mM)
(100 nanomoles Vivo-MO)	0.20 mL	0.5 milliMolar (mM)
300 nanomoles	0.30 mL	1.0 milliMolar (mM)
(400 nanomoles Vivo-MO)	0.80 mL	0.5 milliMolar (mM)
1000 nanomoles	1.00 mL	1.0 milliMolar (mM)
(2000 nanomoles Vivo-MO)	4.00 mL	0.5 milliMolar (mM)

Oligo Concentrations

Typical effective concentrations of standard Morpholino oligos in various systems:

Test system	Oligo concentration
Electroporation in cultures	1 μM to 10 μM (in delivery solution)
Endo-Porter[(a)] in cultures	1 μM to 10 μM (in medium)
Scrape-loading[(b)] in cultures	1 μM to 20 μM (in medium)
Microinjection into oocytes	Inject 1 to 10 nanoliters of 1 mM oligo into 1 μl oocyte to give 1 to10 μM final concentration in oocyte
Cell-free translation system[(c)]	100 nM to 1000 nM (in lysate)

(a) Endo-Porter solution delivers Morpholino oligos into the cytosol of cells efficiently and uniformly by releasing oligos from endosomes.
(b) Morpholino oligos may be loaded into the cytosol/nuclear compartment of adherent cells by adding oligo to the medium and then scraping the cells from the plate (see: Antisense and Nucleic Acid Drug Dev. **6**, 166 (1996)).
(c) See: Antisense and Nucleic Acid Drug Dev. **7**, 63 (1997)

Typical effective concentrations of Vivo-Morpholino oligos in various systems:

Test system	Oligo concentration and/or dose
Cell bathing in cultures	1-10 μM in nutrient medium
Injection into mouse tail vein	12.5 (mg oligo)/(kg mouse) per day
Injection into mouse intraperitoneal	12.5 (mg oligo)/(kg mouse) per day

Cell Delivery Protocols

Upon request GENE TOOLS will provide protocols for Endo-Porter or scrape delivery with cell scrapers. Copies of these protocols are normally shipped with orders which include these products.

QUANTITY

Standard Morpholino oligos

Typical package size for a classic Morpholino oligo is:
300 nanomoles (about 2.5 mg or 75 OD units for 25-mer).
Larger amounts available (1000 nanomole, 6000 nanomole, 1g, etc.).

Vivo-Morpholino oligos

Typical package size for a Vivo-Morpholino oligo is:
400 nanomoles (about 4 mg or 100 OD units for 25-mer).
Larger amounts available (2000 nanomole, 10000 nanomole, 1g, etc.).

Note: The quantities above are the measured and delivered amounts of lyophilized, sterile Morpholino oligos.