Using Morpholinos
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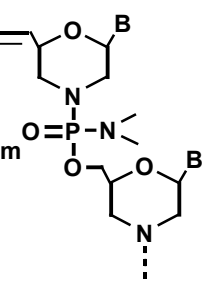

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MSDS are posted at: www.gene-tools.com/MSDS

USE

Oligo state on shipment and storage

Morpholino are shipped as pre-quantitated, sterile, salt-free, lyophilized solid in a glass vial. Store lyophilized oligos at room temperature.

Making a Stock Solution of a Morpholino

We recommend making a ≤1 mM Morpholino stock solution by adding sterile distilled water or PBS or a buffer (e.g. Danieau buffer or Ringer's solution). Morpholinos are not degradable by enzymes such as RNase or DNase. Avoid using diethyl pyrocarbonate (DEPC) treated RNase free water, as any residual DEPC could potentially damage Morpholinos.

To make a 1 mM stock solution, add the appropriate amount of solvent to the lyophilized oligos and immediately vortex vigorously for 2 to 5 minutes to ensure the oligos are fully dissolved.

Morpholino Amount	Solution Volume
100 nmol	100 µl
300 nmol	300 µl
1000 nmol	1000 µl

If your oligo does not dissolve easily during stock preparation, heat the vial to 65°C for 10–30 minutes, vortexing vigorously a few times during the process. If

dissolution remains incomplete, autoclave the stock solution to ensure full solubilization.

GENE TOOLS suggests storing Morpholino stock solutions at room temperature in the original 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.

Morpholinos are very chemically stable, but they can come out of solution either as complexes in suspension or by associating with the surface of a container. The oligos should be heated before use to help them dissolve (65°C for 5 minutes and vortex, or for more difficult solutions you can autoclave the solution on liquid cycle). If you have any questions about the biological activity of a Morpholino, check the solution concentration to see if the actual concentration matches your theoretical calculations using this protocol: https://www.gene-tools.com/sites/default/files/determining_concentration.pdf

Oligo Concentrations

Typical effective concentrations of 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 culture medium)
Microinjection into oocytes	Inject 1 to 10 nL of 1 mM oligo into 1 μl oocyte to give 1 to 10 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)

Cell Delivery Protocols

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

QUANTITY

Morpholinos

Typical package size for a 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.).

Using Vivo-Morpholinos and PPMOs

Oligo state on shipment and storage
Vivo-Morpholinos and PPMOs are shipped as sterile lyophilized solids. Store lyophilized oligos at room temperature.

Making a Stock Solution

A Vivo-Morpholino or a PPMO oligo is delivered as a pre-quantitated, sterile, salt-free, lyophilized solid in a glass vial. We recommend making a ≤0.5 mM stock solution in sterile saline or PBS. Like Morpholinos, Vivo-Morpholinos and PPMOs are not degradable by enzymes such as RNase or DNase. Avoid using diethyl pyrocarbonate (DEPC) treated RNase free water, as any residual DEPC could potentially damage Morpholinos. Delivery dyes with negative charges might inhibit Vivo-Morpholino or PPMO activity.

To make a 0.5 mM oligo stock solution, add the appropriate amount of solution to the lyophilized oligos and immediately vortex vigorously for 2 to 5 minutes to ensure the oligos are fully dissolved.

Oligo Amount	Solution Volume
100 nmol	200 µl
400 nmol	800 µl
2000 nmol	2000 µl

If your oligo does not dissolve easily during stock preparation, heat the vial to 65°C for 10–30 minutes, vortexing vigorously a few times during the process. If dissolution remains incomplete, autoclave the stock solution to ensure full solubilization. Do not autoclave a Vivo-Morpholino or PPMO more than once.

GENE TOOLS suggests storing Vivo- Morpholino or PPMO stock solutions at room temperature in the original GENE TOOLS vial. It is best to keep stocks at 0.5 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 heating the vial to 65°C for 10–30 minutes followed by vortexing.

Oligo Concentrations

The maximum tolerated dosage of Vivo-Morpholino or PPMO can vary depending on factors such as age, genetics, the animal model and injection method used. As a result, conducting a dosage titration experiment is highly

recommended. To ensure accurate dosing in animals, it is highly recommended to check the stock solution concentration using the following protocol: https://www.gene-tools.com/sites/default/files/determining_concentration.pdf

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

Test system	**Oligo concentration and/or dose**
Cell cultures with 0-1% serum	0.1-0.5 µM
Cell cultures with 2-5% serum	1-5 µM
Cell cultures with 5-10% serum	5 -10 µM
Mouse: I.V. Injection	12.5 mg/kg per day
Mouse: I.P. Injection	12.5mg/kg per day
Mouse: ICV injection	0.1-0.4µl of 0.5mM solution
Mouse: local injection	1-10 µl of 0.5mM solution

QUANTITY

Vivo-Morpholinos

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.).

PPMOs

Typical package size for a PPMO 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.