

Morpholino Oligomers

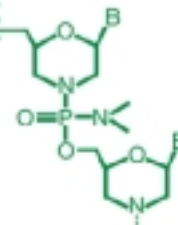
Essential Information

Ordering and Using Morpholinos

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GENE TOOLS, LLC

Website: www.gene-tools.com
 Phone: (541) 753-8330
 Fax: (541) 753-8360
 Mail: P.O. Box 1186
 Corvallis, OR 97339
 USA



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USE

Preparation of Stock Solution

Each oligo is delivered as a prequantitated, sterile, salt-free, lyophilized solid in a glass vial. **For mammalian tissue culture work, we suggest that you make a 0.5 milliMolar stock solution by adding a suitable buffer or sterile water to the vial and vortexing briefly. For developmental studies, we suggest that you make a 1 milliMolar stock solution by adding a suitable buffer (preferred) or sterile water (less preferred). Steve Ekker has determined that for Zebrafish, 1x Danieau buffer works well (58 mM NaCl, 0.7mM KCl, 0.4 mM MgSO₄, 0.6 mM Ca(NO₃)₂, 5.0 mM HEPES pH 7.6). For Xenopus, 0.1X Ringer's Solution works well.**

***** We recommend freezing Morpholino oligo stock solutions to avoid possible concentration increases due to evaporation when lids do not give an airtight seal. *****

Stock solutions of Morpholino oligos for: cell-free translation, scrape loading and special delivery		
Amount of Morpholino	Volume of sterile water	Resulting stock concentration
100 nanoMoles	0.20 mL	500 microMolar (μM)
300 nanoMoles	0.60 mL	500 microMolar (μM)
1000 nanoMoles	2.00 mL	500 microMolar (μM)

Stock solutions of Morpholino oligos for: microinjection into oocytes		
Amount of Morpholino	Volume of buffer or sterile water	Resulting stock concentration
100 nanoMoles	0.1 ml	1.0 milliMolar (mM)
300 nanoMoles	0.3 ml	1.0 milliMolar (mM)

Solubility of oligos can vary with their sequences. In most cases Morpholinos are soluble at over 10 milliMolar. Check to be sure that the oligo is completely dissolved when making 1 mM solutions. If some solid remains, swirl the stock solution for several minutes in hot tap water.

Oligo Concentrations

Typical effective concentrations of Morpholino oligos in various test systems are given below:

Test system	Oligo concentration
Cell-free translation system ^(a)	100 nM to 1000 nM (in lysate)
Scrape-loaded cells ^(b)	1 μ M to 20 μ M (in media)
Special delivery to cells ^(c)	1 μ M (in delivery solution)
Microinjection into oocytes	Inject 1 to 10 nanoliters of 1 mM oligo into 1 μ l oocyte to give 1 to 10 μ M final concentration in oocyte

Notes:

(a) See: Antisense and Nucleic Acid Drug Dev. **7**, 63 (1997)

(b) Morpholino oligos may be quickly and efficiently 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)). Scrape-load delivery is faster, easier, cheaper and less damaging to cells than the liposome delivery schemes commonly used for delivering phosphorothioates and other anionic antisense types.

(c) The Special Delivery EPEI system delivers Morpholino oligos into the cytosol of non-adherent and adherent cells quickly, efficiently, and uniformly using ethoxylated polyethylenimine (EPEI) complexed ionically with prepared Morpholino/DNA duplexes. For more information visit our website (www.gene-tools.com), or call and ask for customer support (541-753-6330).

QUANTITY AND DELIVERY

Oligos are delivered prequantitated, freeze-dried, salt-free and sterile. Our package sizes are:

300 nanoMoles (approximately 2.5 mg or 75 OD units) Morpholino oligo
 300 nanoMoles Special Delivery oligo w/EPEI solution and pre-paired DNA
 Larger quantities (1000 nanoMole, 6000 nanoMole) are also available.

Note: The quantities above are the exact delivered amounts of lyophilized, sterile Morpholino oligos. The 300 nanoMole quantity is roughly the same amount one receives from a typical 1 microMolar scale order of DNA or S-DNA.

We accept Master Card, Visa and American Express credit card orders. Morpholinos are typically shipped two to three weeks after ordering.

Domestic Shipping

Customers in the US will be billed \$20 for shipping and handling. U.S. orders are typically shipped FedEx second day.

International Shipping

We request that international customers provide us with a FedEx account number. We will not bill shipping and handling for those orders; FedEx will bill international customers directly. If available, provide us with a tariff/tax exemption number (VAT#) for your country's customs; otherwise FedEx will add tariffs and taxes to your shipping bill. Contact us by Email if other arrangements are necessary.

ORDERING

For faster, more reliable service and an automatic confirmation of your order, we strongly recommend that you order ONLINE. If this method is unavailable, you may also place your order by FAX or mail. A downloadable form for ordering by FAX or mail is available at our website: www.gene-tools.com. Click on "Ordering" for access to the online order form or the downloadable order form in a PDF format.

Please include a PO number and a telephone number with your order. Be sure to include both a shipping address and a billing address.

SELECTING MORPHOLINO ANTISENSE OLIGOS

Free Oligo Design Service

GENE TOOLS will design appropriate Morpholino antisense sequences for you at no extra charge. In order to use this free design service to order an oligo for blocking protein translation, either:

- provide the GenBank or EMBL accession number for the gene you wish to study; or,
- provide us with the first 25 bases of the coding sequence for the mRNA encoded by the gene of interest and include as much 5' untranslated sequence as is available. Please indicate the true AUG translational start site by putting it in parenthesis and underlining it.

If you wish to block nuclear processing events such as capping, splicing, polyadenylation, or nuclear to cytosol transport you should provide us with the sequence of the pre-mRNA and indicate the specific site you wish to block.

Customer-selected Morpholino Oligo Sequences

Customers who wish to select their own oligo sequences should refer to the targeting guidelines on our web site (www.gene-tools.com).

Note: Morpholino oligos block translation by steric blocking of the translation initiation complex, unlike RNase-H competent antisense oligos (DNA, S-DNA) which prevents translation by degradation of the mRNA. Targeting Morpholinos is generally different from targeting RNase-competent antisense types and the same oligo sequence may not work for both systems. Morpholino oligos containing special sequences such as CpG (where the C is not methylated) and tetra-G do not exhibit the undesirable non-antisense effects often seen with DNA and phosphorothioate oligos.

Suggested Morpholino Control Oligos

We provide 100 nanoMole vials of a standard control oligo at reduced prices, available with or without 3' fluorescein and/or the Special Delivery system. This is the least expensive option for a Morpholino control, but will generally not match the base composition of your experimental oligo.

For typical antisense studies we recommend as a control the invert of the antisense sequence. Many also use the sense sequence as a control:

Antisense: 5'-AAA CCC GGG TTT ACG

Controls:

 Invert of antisense: 5'-GCA TTT GGG CCC AAA

 Sense: 5'-CGT AAA CCC GGG TTT

For rigorous specificity studies, antisense sequences with 4 mispairs appropriately distributed along the sequence provide a much more stringent and realistic assessment of sequence specificity, as illustrated below:

Antisense: 5'-AAA CCC GGG TTT ACG AAC CGG TTT A

Mispaired control: 5'-AAA GCC GAG TTT ACG AAC AGG ATT A

Note: In rigorous studies of sequence specificity using such mispaired controls, Morpholino oligos are found to exhibit high specificity over a concentration range several hundred fold greater than the narrow concentration range wherein Phosphorothioate oligos achieve reasonable sequence specificity (see: Antisense and Nucleic Acid Drug Dev. 7, 63 & 187 (1997)).

CELL DELIVERY PROTOCOLS

GENE TOOLS will provide protocols for Scrape Delivery and Special Delivery upon request. Copies of these protocols are normally shipped with orders when orders include cell scrapers and/or the Special Delivery formulation.

DISCLAIMER

While our and others' experience indicate that Morpholino oligos typically outperform phosphorothioates in cell-free systems and greatly out-perform them in scrape-loaded cells, nonetheless, because of the great variability among genes and cells, GENE TOOLS makes no warranty as to the performance of either GENE TOOLS-designed or customer-selected Morpholino oligos in any given biological system.