Morpholino Oligomers Essential Information

Ordering and Using Morpholinos

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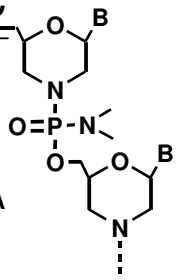

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USE

Preparation of Stock Solution

Each oligo is delivered as a prequantitated, sterile, salt-free, lyophilized solid in a glass vial (except Vivo-Morpholinos, which are delivered in solution). We recommend making a 1 mM stock solution in distilled water, from which Morpholinos can be removed by lyophilization if needed. However, you can make a stock solution in a buffer (e.g. Danieau buffer or Ringer's Solution). Like other oligos, Morpholinos can be damaged by DEPC.

Morpholino oligo **stock solutions may be stored at room temperature, chilled or frozen**. Working stocks can be kept at room temperature or 4 °C. To dissociate aggregates that may form on freezing, after thawing heat the solution for 5 minutes at 65 °C and allow to come to room temperature prior to use.

Stock solutions of Morpholino oligos		
Amount of Morpholino	Volume of sterile water	Resulting stock concentration
100 nanomoles	0.10 mL	1.0 milliMolar (mM)
300 nanomoles	0.30 mL	1.0 milliMolar (mM)
1000 nanomoles	1.00 mL	1.0 milliMolar (mM)

Solubility of oligos can vary with their sequences. In some cases Morpholinos are soluble at over 10 milliMolar, but we suggest keeping stocks at or below 2 mM and recommend 1 mM. **Check to be sure that the oligo is completely dissolved when making stock solutions**. If some solid remains, heat the vial containing the stock oligo solution at 65 °C for 5 to 10 minutes and briefly vortex.

Oligo Concentrations

Typical effective concentrations of Morpholino oligos in various systems:

Test system	Oligo concentration
Electroporation[(a)] in cultures	1 μM to 10 μM (in delivery solution)
Endo-Porter[(b)] in cultures	1 μM to 10 μM (in medium)
Scrape-loading[(c)] 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[(d)]	100 nM to 1000 nM (in lysate)

(a) Electroporation protocol:
http://www.gene-tools.com/files/Amaxa_Morpholino_Oligos_2008-04.pdf
(b) Endo-Porter solution delivers Morpholino oligos into the cytosol of non-adherent and adherent cells efficiently and uniformly by releasing oligos from endosomes.
(c) 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)).
(d) See: Antisense and Nucleic Acid Drug Dev. **7**, 63 (1997)

Cell Delivery Protocols

Upon request GENE TOOLS will provide protocols for Scrape Delivery, Endo-Porter or Vivo-Morpholinos. Copies of these protocols are normally shipped with orders which include these products.

QUANTITY AND DELIVERY

Oligos are delivered prequantitated, freeze-dried, salt-free and sterile.
Typical package size: 300 nanomoles (about 2.5 mg or 75 OD units for 25-mer).
Larger quantities (1000 nanomole, 6000 nanomole, 1g) are also available.

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

Domestic Shipping and Handling

A $15 handling charge is applied to all orders. Customers in the US will be billed $10 for shipping via 2-day FedEx service. FedEx overnight service is available for an additional $10 charge and can be requested on the order form or in the notes section when ordering online.

International Shipping and Handling

A $15 handling charge is applied to all orders. We will bill $50 shipping for international orders. We request that international customers provide us with a FedEx account number for duties, taxes and tariffs. If available, provide us with a tariff/tax exemption number (VAT#) for your country's customs; otherwise FedEx will charge you for duties, taxes and tariffs. Contact us by email if other arrangements are necessary.

ORDERING

For the fastest and most reliable service with an automatic confirmation of your order, we strongly recommend that you order online at the Gene Tools website. 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 in PDF format at our website: www.gene-tools.com. Click on "Ordering" for access to the online order form or the downloadable order form.

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

We accept Master Card, Visa and American Express credit card orders.

Morpholinos are typically shipped within 10 days to two weeks after ordering.

SELECTING MORPHOLINO ANTISENSE OLIGOS

Free Oligo Design Service

GENE TOOLS will design appropriate Morpholino antisense sequences for you at no extra charge. You can submit sequence online at:
http://www.gene-tools.com/Oligo_Design

In order to use this free design service for an oligo to block protein translation, either:

A. **For translational blocking oligos**, provide a GenBank or EMBL accession number for your sequence, **OR** provide the actual 5' UTR sequence through the 25th base of the coding region. **Please mark the start codon with parenthesis ( )** and provide ONLY sequence upstream of the 25th base of coding sequence. Sequence downstream of the 25th base can not be used for translational blocking.
B. **For splice blocking oligos**, please provide 50 bases of exon-intron or intron-exon boundary sequence with exon sequence in UPPER CASE and intron sequence in lower case.
C. **For miRNA inhibiting oligos**, provide the miRBase ID of the miRNA and the desired target site (for details, see http://www.gene-tools.com/node/31).

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) or siRNA, which prevent translation by degradation of the mRNA. Targeting Morpholinos is generally different from targeting with these other oligo types and the same oligo sequence may not work for all of these systems.

Suggested Morpholino Control Oligos

Control experiments involving one or more control oligos are generally performed along with experiments using the targeted custom-sequence oligo. A set of controls usually involves a negative control and a specificity control.

Negative controls: We offer two pre-made negative control sequences: the Standard Control (with or without fluorescent moieties) and the Oligo-N. 100 nanomole vials of these oligos are available at reduced prices. While the standard oligo and the oligo mixture perform very well as negative controls, they will generally not match the base composition of your experimental oligo.

If a negative control oligo with the same base composition as the targeted custom-sequence oligo is desired, we recommend the invert of the antisense sequence as a negative control.

Antisense: 5'-AAA CCC GGG TTT ACG

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

Specificity controls: Specificity control oligos are usually either second non-overlapping targeted oligos or are five-mispair oligos.

Experiments using second non-overlapping targeted oligo are usually one of three types.

The two non-overlapping translation-blocking oligos experiment: This experiment compares the phenotype induced by injection of two different oligos targeted to block translation of the same mRNA. If both sequences induce the same phenotype, that supports the hypothesis that the observed phenotype is due to knockdown of the targeted gene.

One splice blocker, one translation blocker: This uses a splice blocking Morpholino to produce the same phenotype as the translation blocking oligo. It is crucial to determine which exon to target in order to knock down the activity of the protein and phenocopy the translation blocker's effect.

Two splice blockers: In the two-splice-blocker experiment, the same exon is targeted in separate experiments by a splice donor blocker and a splice acceptor blocker. If both of the oligos individually cause clean excisions of the targeted exon, the phenotypes induced by the two oligos should be identical and, again, the hypothesis that the phenotype was triggered by specific excision of the exon is supported.

For rigorous specificity studies, antisense sequences with 5 mispairs appropriately distributed along the sequence provide a stringent and realistic assessment of sequence specificity. A typical mispair control is illustrated below.

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

Mispaired control: 5'-AAA **g**CC **c**GG TTT A**g**G AAC C**c**G T**a**T A

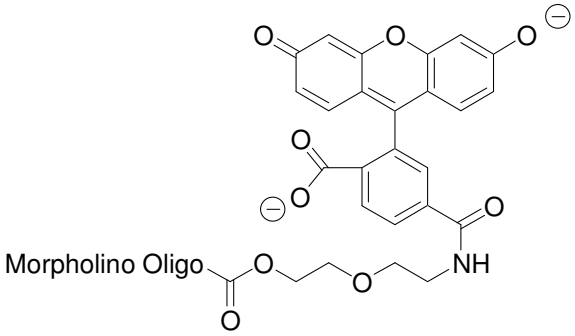
End Modifications of Morpholino Oligos

The mass of a Morpholino oligo which is listed on the oligo's Product Information Sheet includes the mass of the end modification.

3'-Carboxyfluorescein

Green-emitting fluorescent tag
Mass of end modification: 490 Daltons
End of production number contains -F
Example: 12-04Apr05A-F

Excitation peak: 501.5 nm
Emission peak: 524.5 nm

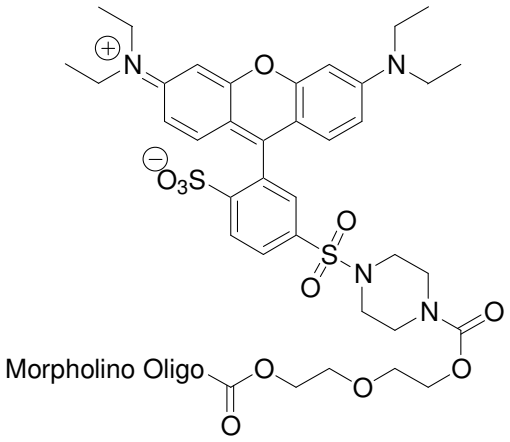


3'-Lissamine

(sulforhodamine B)

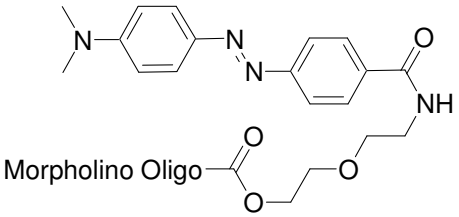
Red-emitting fluorescent tag
Mass of end modification: 785 Daltons
End of production number contains -L
Example: 13-04Apr05A-L

Excitation peak: 575.0 nm
Emission peak: 593.0 nm



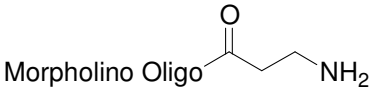
3'-Dabcyl

Fluorescence quencher
Mass of end modification: 383 Daltons
End of production number contains -Q
Example: 14-04Apr05A-Q



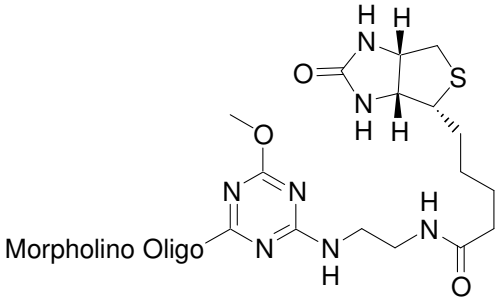
3'-Primary Amine

Functional group for linking compounds
Mass of end modification: 72 Daltons
End of production number contains -A
Example: 15-04Apr05A-A



3'-Biotin

Affinity tag for avidin or streptavidin
Mass of end modification: 394 Daltons
End of production number contains -B
Example: 16-04Apr05A-B

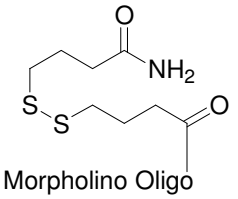


End Modifications of Morpholino Oligos

The mass of a Morpholino oligo which is listed on the oligo's Product Information Sheet includes the mass of the end modification.

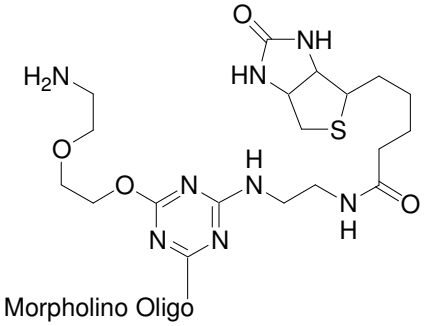
3'-Disulfide

Functional group for linking compounds.
Mass of end modification: 220 Daltons
End of production number contains -S
Example: 12-04Apr05A-S



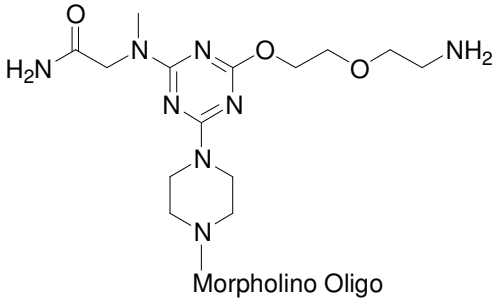
3'-Amine plus biotin

Mass of end modification: 467 Daltons
End of production number contains -AB
Example: 13-04Apr05A-AB



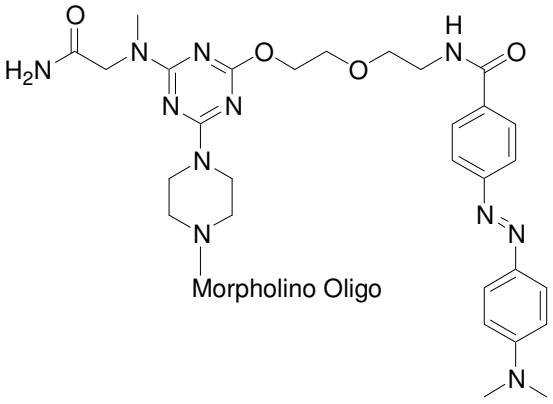
5'-Primary Amine

Functional group for linking compounds.
Mass of end modification: 266 Daltons
If the 3' end is not otherwise substituted, the terminal morpholine nitrogen is capped with a 3'-acetyl, with mass: 43 Daltons
End of production number contains -5A
Example: 15-04Apr05A-5A



5'-Dabcyl

Fluorescence quencher
Mass of end modification: 517 Daltons
End of production number contains -5Q
Example: 14-04Apr05A-5Q



DISCLAIMER

While our and others' experience indicate that Morpholino oligos typically outperform other antisense structural types by a wide margin, 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.