

A Brief Introduction to Morpholino Antisense

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Morpholino oligos block sites on RNA to obstruct cellular processes. A Morpholino oligo specifically binds to its complementary target site to block access of cell components. Morpholinos can inhibit translation, can redirect splicing, or can inhibit activity, maturation or target access of a miRNA.

Translation Blocking: By blocking the translation initiation complex, Morpholinos can knock down translation of many target sequences completely enough that, after waiting for existing protein to degrade, the protein's band disappears from Western blots. Unlike many antisense types (e.g. siRNA, phosphorothioates), Morpholinos translation blockers do not cause degradation of their RNA targets; instead, they prevent translation of the target RNA until that RNA is degraded by normal nuclease activity. Inhibition of translation by a Morpholino should be assayed by immunoblotting. Because the mRNA is not quickly degraded, RT-PCR is not a suitable assay.

Splice Blocking: Used to block sites involved in splicing pre-mRNA, Morpholinos can modify splicing, usually causing targeted exon deletions or intron insertions. This activity can be assayed by RT-PCR, with successful splice-modification visualized on electrophoretic gels as either shifts in mass or disappearance of the RT-PCR product (a band may disappear due to nonsense-mediated decay of the splice-modified transcript).

miRNA Blocking: A Morpholino bound to the guide strand of a miRNA can inhibit its activity; a Morpholino bound to the Drosha processing site of a pri-miRNA or the Dicer processing site of a pre-miRNA can inhibit miRNA maturation. A Morpholino bound to the mRNA target of a miRNA can protect the mRNA from the activity of the miRNA.

Like all gene knockdown reagents, Morpholinos must be actively delivered into cells. For delivery of Morpholinos into cultured cells we recommend electroporation and our Endo-Porter delivery reagent (an endosomal release agent). For embryos, Morpholinos are typically microinjected. For adult animals, we recommend Vivo-Morpholinos.

A Morpholino oligo is radically different from DNA or RNA, with morpholine rings replacing the ribose or deoxyribose sugars and non-ionic phosphorodiamidate linkages replacing the anionic phosphates. Each morpholine ring positions a DNA base (A,C,G,T) so that a 25-base Morpholino oligo strongly and specifically binds to its complementary 25-base target in a strand of RNA. Because the Morpholino oligo's backbone is not recognized by cellular enzymes or signaling proteins, it is completely stable to nucleases and does not trigger an innate immune response through the toll-like receptors.

We encourage you to bring a more precise and powerful tool to bear on your experimental challenges. Call our Ph.D.-level customer support group at GENE TOOLS to get started with Morpholinos in your studies. Phone: (541) 929-7840 ext.1.

Some General Advice for Morpholino Experiments

Solubility and Morpholinos

Keeping Morpholino **stock solutions at or below 1 millimolar** avoids most solubility problems. Though Morpholino oligos are far more soluble than other non-ionic antisense (such as PNAs), Morpholinos with high G content (>30%) can be poorly soluble. Lissamine tags can decrease Morpholino solubility.

Chilling Morpholino solutions in an ice bath may cause the Morpholino to precipitate. DNA, RNA, and most gene knockdown reagents are typically stored in an ice bath during experiments to minimize degradation by nucleases. Because Morpholinos are completely resistant to enzymatic degradation and aqueous solutions are stable indefinitely at room temperature, we recommend that you keep your solutions of Morpholinos at room temperature both on the bench and in storage.

For long-term storage, your Morpholino stock solutions can be **stored at room temperature** in solution or, even better, lyophilized. Room temperature storage favors the solution state (don't let them dry out). Before using stock solutions, we recommend that you heat the oligo solution at 65 °C for 10 minutes and vortex to completely dissolve the Morpholino before use. Solutions of unmodified Morpholino oligos in water can be autoclaved.

These precautions are more than needed for most sequences, but their routine use helps to avoid unpleasant surprises in the event you encounter a sequence with low solubility.

Assessing delivery

As with all gene knockdown agents, delivery of Morpholinos to the cytosol is crucial. Electroporation or our Endo-Porter delivery reagent offer effective delivery into most cultured cell types. You can assess whether you are getting good delivery into the cytosol of your cells by using a fluorescent-tagged Morpholino. We recommend you use our inexpensive carboxyfluorescein-tagged Standard Control oligo for this purpose.

When using a fluorescent Morpholino to confirm delivery, a concentration of 10 μM Morpholino oligo in the culture medium is high enough that, after 16 hours with Endo-Porter, the fluorescent-tagged Morpholino should be visible with a fluorescence microscope as dim diffuse fluorescence in the cytosol. Observe live (unfixed) cells with inverted fluorescence microscope. Use a dry objective lens with the highest available numerical aperture to gather as much light as possible from the cells. Successful delivery appears as diffuse fluorescence throughout the cytosol. In contrast, punctate fluorescence usually indicates that the oligo is trapped in endosomes. Ignore punctate spots and look for the diffuse cytosolic fluorescence.

For most functional experiments with a custom-sequence Morpholino, a concentration of only 1 to 5 μM in the medium is sufficient to achieve biological activity of the Morpholino.