Long-term Storage of Morpholino Oligos

GENE TOOLS recommends either of two storage methods for Morpholinos: store 1 mM aqueous solution at room temperature in a sealed vial or store freeze-dried.

For some Morpholino sequences in solution, the antisense activity of the solution decreases over time; storing at room temperature and heating before use can help avoid this. Tests on oligo solutions that decreased in activity show that the Morpholinos do not degrade, but they can come out of solution by associating with the inside walls of containers or by forming complexes. Decrease in activity can occur faster if oligos are put through freeze-thaw cycles or stored at reduced temperature. Heating to 65°C for 10 minutes can sometimes restore oligo activity. If that is not sufficient heat, in some cases autoclaving has restored activity (be sure to disable the vacuum dry cycle or you will lose liquid).

GENE TOOLS recommends storing a Morpholino solution at 1 mM in a tightly-sealed vial at room temperature. Storing stocks at higher concentrations can lead to variability in results. If you need a higher concentration for an experiment, you can freeze-dry the oligo and dissolve it at a higher concentration for injections, but we recommend you do not store the oligo at the higher concentration. For room temperature storage the seal of the lid to the vial is important, as oligos which have dried out in a vial can be difficult or impossible to completely re-dissolve. Minimize head-space above the oligo solution by using the smallest vial you can. It is good practice to keep the oligos in the dark, as Morpholinos may be ordered with fluorescent tags and fluorescent materials can bleach with exposure to light. Keeping the oligo vials in a humid environment, such as a sealed jar containing a small beaker of water, can decrease evaporation if the seal of the oligo vial is not airtight.

<u>Freeze-drying</u>

If the oligo is to be stored for a long time (many months to years), GENE TOOLS recommends freeze-drying the oligo. We routinely freeze-dry (lyophilize) Morpholino oligos for storage and shipping. We store all holdback oligos in lyophilized form and have lyophilized oligos that have been stored dry at room temperature for over a decade. When dissolved and used, their activity is like new oligos.

We suggest freeze-drying in small glass vials. If you use a flat-bottomed vial, freeze the samples on an angle so that the ice does not cover the entire bottom of the vial; this allows water vapor to escape if the sample melts slightly before the pressure drops below the triple point of water* (below which liquid water will not form). If vapor forms from water boiling at low pressure and the vapor cannot easily escape, it can blow the frozen Morpholino solution out of the vial. We have found vials with bonded glass inserts in which small (30 µl or less) aliquots can be freeze-dried even if frozen upright†. You'll need thin pipet tips for these small vials with inserts.

To freeze-dry aliquots, first dissolve your oligo to 1 mM in aqueous solution with 0.1% phenol red sodium salt (but see the note on oligo mixtures, below). This is a standard concentration of phenol red used to make the solution visible during microinjections. The colored solution also makes pipeting small volumes easier. Heat the solution to 65°C for 10 minutes, vortex, and check that the oligo has dissolved. Aliquot into small glass vials while the solution is warm. Freeze the aliquots, with flat-bottomed vials tilted to expose some of the vial bottom. Freeze-dry in a lyophilizer. Alternatively, for small (<1ml) volumes you can use a desiccation chamber and a strong vacuum pump, as described below.

Freeze-drying without a lyophilizer

Most practicing biochemists either have or have access to a freeze-dryer so, if you do not, try asking a friendly biochemist for help. An alternative is to use a powerful vacuum pump and an airtight chamber, such as a desiccator. Fit a T-valve in the vacuum path between the pump and the chamber so you can release pressure when finished with the freeze-drying. The samples should be frozen at -40°C or -80°C, rapidly transferred to the chamber and then the vacuum pump should be started. The transfer must be done quickly so that the frozen solution does not start to melt before the chamber air pressure reaches water's triple point. You must use a strong vacuum pump that can reach pressures below the triple point of water* within a minute or two, before any of the ice has melted – an aspirator pump or most lab vacuum pumps are not strong enough. Without a cold trap between the sample and the pump, the water from the ice will end up in the pump oil; watch the pump oil quality. The volume of water from a few tiny tubes shouldn't affect the pump and the water will tend to vaporize from warm pump oil, but if you use this technique often be sure to watch the oil for signs of turbidity, increasing the frequency of oil changes if needed.

Oligo mixtures

Freeze-drying is also a useful technique if mixtures of oligos must be made, as 1 mM oligo solutions can be mixed, freeze-dried, then dissolved in a smaller volume of water so that all the oligos are present at 1mM (or higher) in the mixture. In this case you might not want to add phenol red during initial dissolution of your oligos (or might choose to use a lower dye concentration) as the concentration of phenol red will be increased through freeze-drying and re-dissolving in a smaller volume.

Notes

Thanks to Prof. Steve Ekker for telling us of his success with aliquoting and lyophilizing Morpholinos in his lab.

* Triple point of water: 6.1 mBar 4.6 mm Hg 0.0060 atm

† Small vials with glass inserts:
Supelco catalog number 29391-U
Certified QSert™ Clear Glass Screw Thread Vial, 300µl