IDT® INTEGRATED DNA TECHNOLOGIES

Advice for Resuspension of your Oligo

Appearance of the Pellet

Always centrifuge your pellet briefly before opening the tube as oligo pellets may become dislodged during shipping.

Pure, dried oligo can be in either of **two forms:**

1. **White powder:**
   The oligo froze during lyophilisation and the pellet should dissolve instantaneously.
2. **Clear film:**
   The oligo didn't freeze while lyophilising, with the result that the pellet will take longer to dissolve. The tube may require vigorous vortexing to resuspend completely; heating briefly at 55ºC will speed up the process, but is usually unnecessary.

Resuspending dry oligos- IDT recommends:

Dissolve your stock oligo in concentrated TE (10mM Tris pH 8.0, 1mM EDTA) rather than water.

Resuspension calculations can be made using yield information contained on IDT product specification sheets and on the oligo tube. There you will find the actual yield of the oligonucleotide synthesis in three forms: optical density units (OD); mass (in mg); and copy number (in nmole)

1. **To prepare a 100µM stock:**
   µl TE needed = 10 X nanomoles of oligo (as printed on spec sheet)
   example: 500µl TE + 50nmole oligo 100µM stock
2. **To prepare working solutions:**
   Dilute stock 1:10 in water 10µM oligo
   1µl working solution in 20µl reaction volume 0.5µM = 10picomoles primer
   Mass units: use milligram amounts on the spec sheet provided to make the stock solution.

Resuspension buffers:

IDT strongly recommends TE buffer (or some other pH-controlled buffer) for resuspending oligos. Resuspending oligos in water will allow the solution to become acidic, causing acid-nicking of the phosphodiester backbone, and thereby decreasing the half-life of the oligos. pH controlled buffers help maintain an ideal pH for handling oligos (pH 7.5-8.0) which will allow the oligos to remain stable and maintain their structural integrity.

Use of DEPC water is strongly discouraged for several reasons. DEPC (Diethyl Pyrocarbonate) is carcinogenic. It also has a strong affinity for adenosine, and even trace amounts will result in chemical modification of adenine residues. DEPC is reactive with primary amine groups and mercaptans. In addition, DEPC should never be added to buffers that contain TRIS (tris(hydroxymethyl)AMINOmethane). In addition, DEPC is hard to remove and interferes with PCR and other reactions.

Stock solution

Make up small aliquots of stock solution rather than maintaining one large stock. Incomplete thawing of a sample can lead to inconsistent concentrations being pulled from the stock tube, so thaw the stock completely each time a working solution is made up. Repeated freeze/thaw cycles are not good for an oligo, aliquots should be small enough to not thaw the tube more than 3-4 times.

Material can be lost due to absorption of the oligo to the tube if polystyrene tubes are used. The use of polypropylene tubes is preferred.

Resuspension of hard-to-suspend Oligos

Heat the oligonucleotide at 55°C for 1–5 minutes, then vortex thoroughly. If there is still a visible precipitate in the tube, the sample may contain silica which is a by-product of oligo synthesis. It will not affect the performance of the product, and may be removed through filtration or decanting the supernatant.

Oligonucleotide Storage

Long-term storage:
If you would like to use a portion of the oligonucleotide immediately and then store the remaining mass for future use, it is best to resuspend the entire product in TE (Tris-EDTA, pH 8.0) at the desired stock solution concentration. Take a sufficient volume for immediate use from this stock and dilute it to a working stock concentration. The remaining oligonucleotide solution can be treated in one of two ways for subsequent long-term storage. The ideal situation is to dry the DNA down and store it at-20°C. If this is not practical, then the next best thing is to make small aliquots of the stock suspension and store these at-20C. Creating aliquots will allow you to avoid potential contamination from use of a single tube. DNA kept frozen in nuclease-free environment should be stable for years.

Short-term storage:
Never store dilute solutions of DNA at 4ºC for extended periods of time (ie more than one day). In dilute concentrations <1µM, the DNA can adsorb onto the plastic of the tube, changing the concentration of the solution.

Short-term storage of concentrated stock solution of oligonucleotides at 4°C is acceptable as long as this does not exceed 7 months.

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