Handling Guidelines for DNA and RNA Oligonucleotides

Oligonucleotides (oligos) require certain basic storage and handling techniques in order to ensure trouble-free experiments. Proper storage of your oligo will maximize its shelf life, allowing you to get the most use from the oligo. These guidelines will enable you to work easily with your oligo in your application.

Oligo Resuspension and Storage

All DNA oligonucleotides that are provided dry are ready for use upon resuspension, with the exception of Thiol modified oligos. We recommend resuspending oligos in a weak buffer such as TE buffer (10 mM Tris, pH 7.5 - 8.0, 1 mM EDTA, diluted from Sigma T9285). In applications in which TE is not suitable, sterile nuclease-free water (Sigma W4502) may be used. However, high grade water may be slightly acidic and is not recommended for long-term storage. In RNA-related applications, DEPC-treated water may be used, but residual DEPC may inhibit subsequent enzymatic reactions.

siRNA duplexes from Sigma should be resuspended in sterile, nuclease-free water (Sigma W4502). It is not necessary to add buffers when resuspending siRNAs, and after resuspension the siRNAs are ready-to-use.

Single-stranded RNA oligos should be resuspended as described above for DNA oligos.

Preparation of a Concentrated Stock Solution

For all oligos a 100 µMolar stock solution may be obtained by using the following guideline. Take the number of nanomoles (nm) provided (information found on the tube label and/or quality assurance document) and multiply the number of nanomoles by ten. The result provides the number of microliters of liquid (TE, Tris, water or other buffer as required) to add to the tube for reaching a final concentration of 100 µMolar. Note that this is equivalent to a stock solution of 100 pmol/µL. The stock solution may then be further diluted as necessary based upon the application requirements. Store the stock solution at - 20 ˚C in a non-frost-free freezer, and avoid multiple freeze-thaw cycles. Single-stranded RNA oligos should be stored at - 80 ˚C.

Fluorescently Labeled Oligos

If using fluorescently modified oligos, it is important to note that the intensity of a fluorescent signal can often be directly affected by the pH of the solution used for resuspending. A buffered solution such as TE is recommended for the resuspension of fluorescently labeled oligos. Fluorescently labeled oligos should have minimal exposure to light to prevent dye photobleaching. Wrapping the tubes in foil and/or storing in amber colored tubes is suggested.

Oligonucleotide Quantification

The microgram quantity of an oligonucleotide is directly related to its ultraviolet absorbance or optical density (OD) at 260 nm. We take great care to ensure that the OD reading determined for each oligo is accurate. You may choose to re-quantify your oligo for verification prior to use. There is a wide range of devices that might be used to measure absorbance at 260 nm. If using a traditional cuvette-based spectrophotometer, the following procedure applies.

Resuspend the oligonucleotide in 400 µl of water. Remove a 12 µl aliquot (to ensure the volume is within the accurate and reproducible range of micropipettes) from the resuspension and dilute with 988 µl of sterile water. Ensure that the OD reading is within the linear range of accuracy of the spectrophotometer (typically between 0.1 and 1.5 OD units). If the value is outside the linear range, remeasure using a more appropriate dilution for that sample.

We recommend trying 12 µl from the original 400 µl suspension; therefore, the OD of the 12 µl must be multiplied by a dilution factor of 33 (which is 400/12) and then by the original volume in mL (0.400) to determine the OD of the entire oligo sample.

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Sigma calculates µg/OD (A260) based on the extinction coefficient of each base in the oligonucleotide sequence using a nearest-neighbor method. This value is printed on the oligo tube label and QC document that accompanies each shipment. The total number of micrograms of oligo in the 400 µl suspension can be calculated as follows:

Total µg of oligo in original 400 µl = OD reading for 12 µl aliquot x dilution factor 33 x X µg/OD (see tube label)

The quantity of oligonucleotide supplied is expressed in nanomoles on the oligo tube label, along with the µg quantity of the oligo and its molecular weight (MW). Sigma calculates the number of nanomoles using the amount of oligo (in µg) and the molecular weight as follows:

Total nmol = µg of oligo x 1 µmol/MW x 1000 nmol/1 µmol

(Note: 1 mole = 10^3 mmoles = 10^6 µmoles = 10^9 nmoles = 10^{12} pmoles)

For additional information on resuspension and handling of the modified oligos and oligo quantitation, please see our technical resources section at sigma.com/oligos.

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