Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dye labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

Thiol Modified Oligo Disulfide Reduction

For research use only. Not for use in diagnostic procedures for clinical purposes.

Reduction of Thiol Modified oligos

Gene Link synthesized thiol modified oligos (all thiol modified DNA oligos, RNA, siRNA and molecular probes) are shipped lyophilized and as a disulfide protected* form to prevent the formation of dimers. These can be reduced by the end user prior to use.

The preferred method is to reduce thiol labelled oligos using TCEP [Tris (2-carboxyethyl) phosphine hydrochloride; Gene Link Cat.# 40-5116-10]. The oligos can also be reduced by the classical DTT method. Both methods are given below.

TCEP Reduction Protocol

1. Prepare 400 µL of 0.1M TCEP (~3%) solution by adding 80 µL of 0.5M TCEP to 320 µL of sterile RNase free water. Prepare more as required. Preparing fresh 0.1M TCEP dilution is recommended.
2. Add 400 µL of 0.1M TCEP directly to lyophilized thiolated oligos. Vortex to dissolve oligo. Leave at room temperature (RT) for 1 hr to reduce the thiol groups. Vortex intermittently.
3. Add 50 µL of 3M Sodium Acetate pH 5.2 and vortex.
4. Add 1.0 mL of absolute ethanol, vortex and store at -20°C for 20 minutes.
5. Centrifuge at 12K rpm for 10 minutes. Decant ethanol and air dry pellet.
6. Dissolve in 200 µL of sterile RNase free water or choice of buffer volume as required.
7. Determine sample concentration by obtaining an absorbance at 260 nm.

DTT Reduction Protocol

1. Prepare a 100mM DTT solution in sodium phosphate buffer, (pH 8.3 – 8.5). DTT is available as a solid from Sigma-Aldrich (Product No. D9779). For 5 mL of the 100 mM solution, add 77.13 mg of the DTT powder
2. Add 400 µL of 100 mM directly to lyophilized thiolated siRNA. Leave at room temperature (RT) for 1 hr to reduce the thiol groups. Vortex intermittently.
3. Add 50 µL of 3M Sodium Acetate pH 5.2 and vortex.
4. Add 1.0 mL of absolute ethanol, vortex and store at -80°C for 20 minutes.
5. Centrifuge at 12K rpm for 10 minutes. Decant ethanol and air dry pellet.
6. Dissolve in 200 µL of sterile RNase free water or choice of buffer volume as required.
7. Determine sample concentration by obtaining an absorbance at 260 nm.

*Prior to July 20, 2011 all Gene Link supplied thiolated oligos were supplied in a reduced state.

Handling & Storage of RNA Oligos

Follow established stringent RNase free handling conditions. The lyophilized RNA or siRNA duplex should be stored immediately at -20° C. The lyophilized siRNA is stable for ~6 months at -20° C.