INTEGRATED DNA TECHNOLOGIES

Reduction Protocol for Thiol-Modified Oligonucleotides

Thiol-modified oligos ordered from IDT are shipped in their oxidized form, with the sulfur atoms protected by an S=S bond. See our Modifications Page under the "Products" tab on the IDT website to view the protecting group. Thus, thiol-modified oligos need to be reduced before use. Thiol-modified oligos require a reduction step to remove the protecting group from the oligos before they are used. There are several ways to do the reduction:

Note: *The reducing protocol is more successful when performed in a pH-controlled environment. Therefore, we would strongly recommend using a buffer, such as TE Buffer. Also, once treated, any oligonucleotide that is not used immediately should be stored frozen. Over time, the oligo will oxidize and the below procedure will need to be repeated prior to coupling.*

1. **Treatment with solid-phase DTT**
   DTT is available immobilized on acrylamide resin (Reductacryl™, Calbiochem Inc. Cat. No. 233157).
   - Add the Reductacryl in excess to your oligo.
   - Run the reaction through a spin column.
   - You can add the resultant oligo directly to your reaction mix.
2. **Treatment with DTT in liquid phase**
   The oligo can be treated with or stored in DTT. Remove the DTT immediately before use.
   - Make a solution of 100 µM oligo in 10 mM DTT made up in 1X TE.
   - Pass the solution through a large bed volume Sephadex column to remove DTT. *Note: small bed volume spin columns can allow trace DTT to remain with the oligo, which can interfere with subsequent coupling reactions.*
3. **DTT bulk reduction**
   - Reconstitute up to 1 mg oligo in 100 µL 2% TEA (triethylamine), 50 mM DTT
   - Allow to stand at room temperature for 10 min.
   - Remove DTT using 1 of the 3 methods outlined below:
     I. **Extraction and precipitation:**
     Extract 4 times using 400 µL ethyl acetate (layers readily separate; DTT partitions with the ethyl acetate and the DNA partitions in the aqueous phase).
     II. **Acetone precipitation or gel filtration:**
     In a 14 mL tube, add 5:1 acetone solution (2% $LiClO_4$) w/w in acetone:oligo solution. Chill the resulting solution at -20°C for 15 min. Centrifuge the sample at 2500 or 5000 RPM for 10 or 5 min.

respectively. Remove the supernatant and dry the sample under vacuum to remove trace acetone. Remove $LiClO_4$ and other salts by washing the sample with 2–3 mL n-butanol, centrifuge again, and remove the butanol supernatant.

III. **Size exclusion/gel filtration chromatography**:
Load oligo sample on a Sephadex G25F column that has been thoroughly washed with distilled water. Elute the column with water by gravity flow and collect fractions. Measure UV absorbance at 260 nm. The first eluting peak at the void volume is the oligonucleotide. Concentrate fractions using a SpeedVac evaporator.

4. **DTT alternative: TCEP (Tris[2-carboxyethyl] phosphine) treatment**
   - Add TCEP in 100X excess (i.e., 30 mM TCEP to 300 µM oligo)
   - Let it sit for a couple of hours at room temperature to reduce the oligo.
   - You do not need to remove the TCEP from your oligo before using it in your reaction.