Reduction for oligonucleotides with thiol modifications

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REDUCTION FOR OLIGOS WITH THIOL MODIFICATIONS

Oligos with thiol modifications supplied by IDT are shipped in the oxidized form, with the sulfur atoms protected in an S=S bond. Before using the oligos, the S=S bond must be reduced to release the thiol groups. There are several methods available to reduce the S=S bond, as outlined in this protocol.

Notes:

- Since oligos containing thiol modifiers are shipped in their oxidized (disulfide) form, they require chemical reduction by dithiothreitol (DTT) or Tris (2-carboxyethyl) phosphine (TCEP) before each use.
- This protocol is best performed in a pH-controlled environment. We strongly recommend using a buffer, such as TE buffer. After treatment, freeze any oligonucleotide that is not immediately used. If not, the oligo will oxidize over time, and this protocol would need to be repeated before oligo use.
- The structure of the oligo with the protecting group can be understood by reading this DECODED article.

TREATMENT WITH DTT IN LIQUID PHASE

The oligo can be treated with or stored in DTT.

Note: Remove the DTT immediately before use.

1. Make a solution of 100 µM oligo in 1X TE with 10 mM DTT.
2. Pass the solution through a large-bed volume Sephadex® column (GE Healthcare) to remove DTT (See Table 1).

Table 1. Large-bed spin column information. [1]

Description	Ultra-micro	Micro	Macro
Bed volume	37.96 µL	66.42 µL	191.45 µL
Sample volume	10–25 µL	25–75 µL	75–150 µL
Sample amount	3–30 µg	5–60 µg	30–300 µg

Note: Small-bed volume spin columns can allow trace DTT to remain with the oligo, which can interfere with subsequent coupling reactions.

REDUCTION WITH DTT FOR LARGER-SCALE OLIGOS

1. Reconstitute up to 1 mg oligo in 100 µL of 2% TEA (triethylamine), 50 mM DTT and keep at room temperature (15 to 25°C) for 10 minutes.
2. Remove DTT by one of the following 3 methods:
   a. Extract in ethyl acetate
   b. Precipitate in acetone
   c. Use gel filtration chromatography (size exclusion)

Extraction in ethyl acetate

Extract 4 times using 400 µL of ethyl acetate.

Note: Layers readily separate—DTT partitions with the ethyl acetate, and the DNA partitions, in the aqueous phase.

Precipitation in acetone

1. In a 14 mL tube, make a solution of 5 parts acetone with 2% (w/v) $LiClO_4$ to 1 part oligo.
2. Chill the resulting solution at –20°C for 15 min.
3. Centrifuge the sample at 2500 rpm for 10 minutes, or at 5000 rpm for 5 minutes.
4. Remove the supernatant and dry the sample under vacuum to remove trace acetone.
5. Remove $LiClO_4$ and other salts by washing the sample with 2–3 mL of n-butanol.
6. Centrifuge again, then remove the butanol supernatant.

Size exclusion by gel filtration chromatography

1. Load oligo sample on a Sephadex G25F column that has been thoroughly washed with distilled water.
2. Elute the column with water by gravity flow and collect fractions.
3. Measure UV absorbance at 260 nm. The first eluting peak at the void volume is the oligonucleotide.
4. Concentrate fractions using a centrifugal, vacuum evaporator.

OTHER TREATMENTS

TCEP (Tris [2-carboxyethyl] phosphine) (DTT alternative)

1. Add TCEP in 100X excess (i.e., 30 mM TCEP to 300 µM oligo).
2. Keep for 2 hours at room temperature to reduce the oligo.

Note: You do not need to remove the TCEP from your oligo before using it in your reaction.

Treatment with solid-phase TCEP

1. Add 1–2 volumes of Pierce™ Immobilized TCEP Disulfide Reducing Gel (Thermo Fisher Scientific) to your oligo.
2. Run the reaction through a spin column.
3. Add the resultant oligo directly to your reaction mix.

REFERENCES

1. Harvard Apparatus, www.harvardapparatus.com/media/harvard/pdf/Guide+for+Gel+Filtration.pdf. [Accessed 6 May, 2020].

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