

# Dharmacon<sup>TM</sup> TCEP reaction for thiol-modified siRNA/RNA oligonucleotides

This protocol is for the deprotection of thiol-modified siRNA/RNA oligonucleotides

Thiol-modified siRNA and RNA oligonucleotides with the thiol group in a protected or oxidized form prevent the formation of dimers. Prior to use, the thiol groups on the thiol-modified oligonucleotide can be deprotected or reduced using the following protocol.

## Materials required

- Thermo Scientific<sup>TM</sup> Bond-Breaker<sup>TM</sup> TCEP [Tris (2-carboxyethyl) phosphine hydrochloride] solution, neutral pH, 0.5M (Cat #77720)
  - Make 1.0 mL of 3% TCEP solution by adding 30  $\mu$ L of TCEP to 970  $\mu$ L of molecular grade RNase-free water.
- Molecular Grade Water, RNase-free
- 3 M Sodium Acetate
- 200 proof, 99.5% Ethyl Alcohol

## Protocol

1. Add 400  $\mu$ L of 3% TCEP solution to dry oligonucleotide.
2. Vortex sample until it is in solution.
3. Place sample on rocker for 1 hour.
4. Add 50  $\mu$ L of 3 M Sodium Acetate.
5. Add 1.5 mL of 200 proof Ethyl Alcohol.
6. Place in  $-80^{\circ}\text{C}$  freezer for at least 20 minutes.
7. Remove sample from freezer and spin in a micro-centrifuge at  $13000 \times g$  for 20 minutes ( $4^{\circ}\text{C}$ ).
8. Pour the supernatant from the tube into a 2 mL tube and save.
9. Slowly pipette 200  $\mu$ L of 95% Ethanol onto the pellet.
10. Pour the 95% Ethanol from the tube.
11. Dry the sample under vacuum with a lyophilizer or speed-vac.
12. The dry pellet can be resuspended in an appropriately buffered RNase-free solution.
13. Quantify the sample by obtaining an absorbance at 260 nm.
  - If quantity of sample is acceptable, discard supernatant.

## If you have any questions, contact

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