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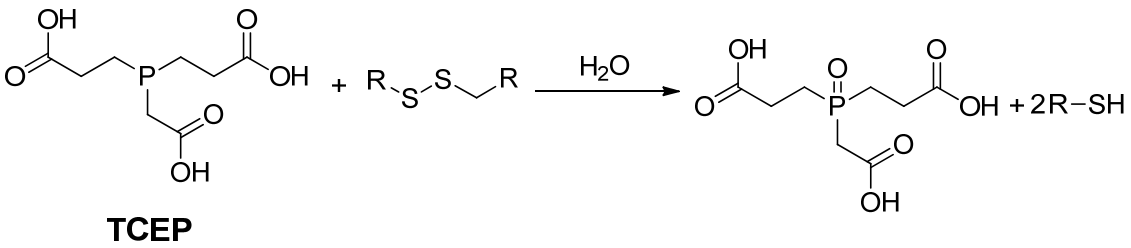
Instruction of reduction reaction using TCEP

Description of TCEP·HCl

TCEP•HCl, Tris(2-carboxyethyl)phosphine hydrochloride
Molecular Weight: 286.64; CAS #51805-45-9
Safety and Storage: Upon receipt store at room temperature in sealed container to prevent oxidation.

TCEP is a potent, versatile, odorless, thiol-free reducing agent with broad application in reduction of disulfide bonds (Figure 1). The unique compound is easily soluble and very stable in many aqueous solutions. The hydrochloride salt (TCEP•HCl, MW 286.64) has a solubility in water of 310 g/L (1.08 M). Being hydrophilic, TCEP is generally very soluble in aqueous buffers at nearly any pH. TCEP has only minimal solubility in organic solvents, including methanol and ethanol. TCEP is stable in aqueous, acidic, and basic solutions. Studies indicate that no change in concentration of TCEP occurs after 24 hour incubation at room temperature in 100 mM HCl, 100 mM NaOH, and most of 50 mM buffer system.



TCEP

Figure 1. Reduction of organic disulfide bonds with TCEP.

TCEP reduces disulfide bonds as effectively as dithiothreitol (DTT), but unlike DTT and other thiol-containing reducing agents, TCEP does not have to be removed before certain sulfhydryl-reactive cross-linking reactions. TCEP selectively and completely reduces even the most stable water-soluble alkyl disulfides over a wide pH range. Reductions frequently require less than 5 minutes at room temperature. TCEP is non-volatile, odorless, and unlike most other reducing agents, is resistant to air oxidation. Compared to DTT, TCEP is more stable, more effective, and able to reduce disulfide bonds at lower pHs.

TCEP effectively reduces disulfide bonds over a broad pH range. A pH range **(1.5<pH<9.0**) is recommended for the reduction reaction.

Procedure for the reduction reaction

Into a buffer solution containing oligos, 10 molar equivalents of TCEP was added under stirring at room temperature. And the mixed solution was stirred at r.t. for about 10 minutes. The more TCEP is used in the reaction, the faster the reduction reaction complete. Generally, S-S bonds on oligos could be reduced into SH group.

Except the oligo-O-$(CH_2)_3$SH group, there is another chemical with thiol group, HS-$(CH_2)_3$OH, formed in this reducing method due to the structure of oligo-O-$(CH_2)_3$-S-S-$(CH_2)_3$OH we provided. This chemical, HS-$(CH_2)_3$OH, should be considered before the next step reaction. For example, in the reaction involving maleimide, we recommended that more maleimide (2-folds regular amount) should be used.

Notes: TCEP is not particularly stable in phosphate buffers, especially at neutral pH. Therefore, if TCEP is to be used in PBS buffers, prepare the working solution immediately before use.