Tris(3-hydroxypropyl)phosphine and Tris(2-carboxyethyl)phosphine hydrochloride: disulfide reducing agents for molecular diagnostics

Properties of Tris(3-hydroxypropyl)phosphine and Tris(2-carboxyethyl)phosphine, hydrochloride

Tris(3-hydroxypropyl)phosphine (TPP or THPP) and Tris(2-carboxyethyl)phosphine, hydrochloride are, water soluble and used as neutral sulfhydryl reducing agents. They have a greater reducing capacity than dithiothreitol (DTT). In addition, THPP and TCEP are suitable for use in immobilized metal affinity chromatography because they do not reduce the metals involved.

Advantages of THPP and TCEP as Reducing Agents

In biological systems, to maintain biological activity, it is important that the thiol groups in peptides and proteins remain in their reduced state. Thiols, such as 2-mercaptoethanol and DTT, are commonly used as disulfide reductants. However, DTT can interfere in the desired reactions of SH groups. It can also be oxidized by metal-affinity columns, and thus must be removed prior to peptide modification or purification.

Trialkylphosphines, on the other hand, exhibit high selectivity for disulfide bonds, but are generally not water soluble and have an unpleasant odor. The high water solubility of THPP and TCEP, therefore, make these two phosphines ideal alternatives to DTT. Unlike DTT, they generally do not need to be removed prior to thiol modification. THPP has the added advantage of being effective over a wide pH range[1].

Applications of THPP and TCEP as Reducing Agents

THPP and TCEP are used to break disulfide bonds within and between proteins in a wide range of biological applications, such as protein cleavage and/or precipitation for molecular diagnostics, and gene chips and microarrays. In fact, THPP and TCEP are both found in numerous enzyme kits for biological processing, such as protein extraction[2], specific amino acid sequence cleavage[3], and protein in-gel tryptic digestion[4].

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Some example literature citations that demonstrate the range of uses for THPP and TCEP include:


**Other Applications of THPP and TCEP**

THPP is also useful for the removal of protecting groups from S-protected cysteins, the deoxygenation of sulfoxides, N-oxides, and other sulfur and nitrogen compounds[5]. It is also a reagent for peptide synthesis, particularly in Mitsunobu and Staudinger reactions[6].

![Staudinger-Wittig reaction](image)

Peptide-bond formation, using Staudinger-Wittig reaction

TCEP, meanwhile, does not impede maleimide attachment, and is suitable as a reagent for the quantitative analysis of sulfides, disulfides, hypochlorite ions, iodine, iodate ions, sulfoxides, N-oxides, azides[7], and the selective reduction of disulfides for organic synthesis[8].

References:

4. Agilent 5188-2749Protein In-Gel Tryptic Digestion Kit Instructions.

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