Technical Data Sheet

DTT

for molecular biology

Order number: **1114**

The reducing agent 1,4-dithiothreitol (DTT), also known as Cleland's reagent, is routinely used in biochemical laboratories. Because of its low redox potential (-0.33 V at pH 7), DTT is able to protect free SH groups (i.e., keep them in the reduced state) and quantitatively reduce existing disulfide bridges (convert them to free SH groups). Upon oxidation, DTT reversibly forms an energetically favored six-membered ring with intramolecular disulfide bonding. The reducing effect of DTT is limited to pH values above 7 since only the negatively charged thiol form is reactive. The thiol groups have pKa values of 9.2 and 10.1.

DTT is readily soluble in a wide variety of solvents including water, ethanol, acetone, ethylate, chloroform and ether.

DTT can be used as an alternative to ß-mercaptoethanol and has several advantages in direct comparison: DTT is generally more stable than 2-mercaptoethanol and has a less unpleasant odor. In addition, it can be used in smaller quantities due to its more favored oxidation product.

Application

DTT has numerous applications in protein chemistry, including the isolation, purification, and characterization of enzymes and proteins, protein assays (activity measurements), and specifically in the study of the role of cysteines in protein structure and function.

At low concentrations, DTT keeps proteins in their active form by preventing the oxidation of sulfhydryl groups to disulfide bridges by atmospheric oxygen. DTT is used accordingly in some reaction buffers to maintain enzymatic activity. In higher concentrations DTT destroys the disulfide bridges in proteins and thus breaks down their tertiary structure. This property is used, for example, in the sample buffer for SDS-PAGE. DTT reduces all disulfide bridges to thiols and breaks intramolecular bonds as well as intermolecular bonds between subunits.

For disulfide bonds that are difficult to access as they are located deep within the protein structure, additional strongly reducing conditions are required for successful reduction. DTT can be readily used in the presence of denaturants such as guanidinium chloride, urea, and SDS.

DTT is also used as a reducing agent for thiolated DNA, preventing the formation of unwanted dimers.

Please note: DTT reduces nickel ions and can accordingly cause problems in the purification of HIS-tagged proteins.

neoFroxx GmbH
Marie-Curie-Str. 3
D-64683 Einhausen

www.neofroxx.com
Phone +49 (6251) 989 24-0
info@neofroxx.com

Preparation of DTT solutions

Stock solution:	1 M in distilled water (1,54 g DTT/ 10 ml). If sterility is required use a filter. Do not autoclave. Store aliquots at -20°C.
Working concentration:	0.1 M for complete reduction of proteins (Sample buffer for SDS-PAGE) 1 – 10 mM to maintain the reduced state of proteins in solution.

Storage

DTT is hygroscopic and sensitive to heat and reacts with oxidizing agents (atmospheric oxygen). The solid is stable for at least 4 years at 2 - 8°C under exclusion of light and humidity. At room temperature, DTT is stable for only a few days in tightly closed containers.

Dissolved in water or buffer, DTT is not stable, so solutions are best prepared fresh. DTT solutions lose activity rapidly and must be frozen in aliquots at -20°C immediately after preparation for long-term storage.

JB06092022