

Product Information

Dithiothreitol Solution

1 M

Product Code **64,656-3**

Store at Room Temperature

CAS# 3483-12-3

Synonyms: Cleland's Reagent, DTT

Product Description

Molecular Formula: $C_4H_{10}O_2S_2$

Molecular Weight: 154.3

This product is a ready-to-use 1 M dithiothreitol (DTT) solution. It is designated as Electrophoresis grade and has been tested for use in an SDS-PAGE sample buffer.

Dithiothreitol (DTT) is used in proteomics applications to maintain sulfhydryl (-SH) groups in the reduced state and for quantitative reduction of disulfide (-S-S-) groups, as described by Cleland in his pioneering work in the 1960's.¹ By reducing the disulfide bonds in a protein sample, the protein can be more effectively fragmented and analyzed.

DTT is a commonly used reagent in buffers because of its ability to reduce oxidation of a protein sample, and thereby, preserve enzymatic activity.² DTT is oxidized to the cyclic disulfide during the reduction of other disulfides in solution. Disulfide reduction is typically complete in minutes at pH 8. Its usefulness stems from its water solubility, reduced odor, and lower toxicity compared to other thiol compounds (2-mercaptoethanol).¹ Typically, a 7-fold lower concentration of DTT (100 mM) is used compared to 2-mercaptoethanol [5% (v/v), 700 mM].

Dithiothreitol is a versatile compound that can be used in many downstream applications. These include SDS-PAGE, chromatography, and modification of cysteine containing compounds. For cysteine modification, it is recommended that the DTT be removed prior to labeling, because the -SH groups of DTT will compete directly with the protein for attachment of thiol reactive labels.²

The concentration of DTT can be quantitatively determined by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). In this procedure, the DTT completely reduces the disulfide bond of the DTNB to produce two molecules of the thiol NTB, which can be measured at 412 nm.³

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

This product is supplied ready-to-use.

Storage/Stability

The unopened product is stable for at least two years at room temperature.

Procedure

SDS-PAGE sample preparation with DTT

1. Dilute the 1 M DTT Solution to 50 mM by adding 50 μ l of the 1 M DTT Solution to 950 μ l of ultrapure water.
2. The SDS-PAGE sample is prepared by combining 5x ProteoGel™ Tris Acetate Sample Buffer (Product Code T 5196), water, the protein sample, and the prepared 50 mM DTT solution. Examples are given in Table 1. Vortex the samples to mix.

Table 1.
SDS-PAGE Sample Preparation

Concentration of Protein Sample (mg/ml)	Volume of Protein Sample for SDS-PAGE Sample (μ l)	50 mM DTT Solution (μ l)	5X Sample Buffer (T 5196) (μ l)	Water (μ l)
2	25	5	10	10
4	12.5	5	10	22.5
5	10	5	10	25
10	5	5	10	30

Amounts are based on a sample volume of 50 μ l with a final protein concentration of 1 mg/ml and a final DTT concentration of 5 mM. The actual concentrations and volumes used will depend upon the protein sample.

3. Boil the samples for five minutes.
4. Allow the samples to cool and then load onto an SDS-PAGE gel.

References

1. Cleland, W. W., Dithiothreitol, a new protective reagent for SH groups. *Biochemistry*, **3(4)**, 480-482 (1964).
2. Getz, E. B., et al., A comparison between the Sulfhydryl Reductants Tris (2-carboxyethyl) phosphine and Dithiotreitol for Use in Protein Biochemistry. *Anal. Biochem.*, **273**, 73-80 (1999).
3. Han, J. C., and Han, G. Y., A procedure for quantitative determination of tris (2-carboxyethyl) phosphine, an odorless reducing agent more stable and effective than dithiothreitol. *Anal. Biochem.*, **220**, 5-10 (1994).

BE/MAM 5/04

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