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ProductInformation

8 M Guanidine Hydrochloride Solution
Buffered, pH 8.5

Product Code **G 7294**
Store at Room Temperature

Product Description

This product is a ready-to-use 8 M guanidine hydrochloride solution buffered at pH 8.5 with 0.05 M bicine. It is ideal for use with affinity tagging procedures such as labeling and modification of cysteine residues. The bicine buffer does not contain primary amines, phosphates, or carboxyl groups, and therefore, is compatible with mass spectrometric procedures.

Guanidine hydrochloride is commonly used as a denaturant, because of its ability to break hydrogen bonds between amino acid residues. By breaking these bonds, the 3D conformation of the protein is unfolded and the aqueous solubility of the protein is greatly increased. Once denatured, the protein can be easily reduced, modified, or analyzed, in a variety of procedures. Further processing of the sample can allow the proteins to be analyzed by other common protein analysis methods including mass spectrometry, electrophoresis, and enzymatic digests.

Guanidine hydrochloride has typically been used for the isolation of RNA, to denature globular proteins, and for protein refolding studies. It can also be used to facilitate the generation of tryptic peptides for analysis of complex protein samples.

Reagents Required But Not Provided For Optional Procedure

200 mM Tributylphosphine Solution (TBP, Product Code T 7567, supplied ready-to-use)

0.5 M Iodoacetamide Solution (prepared from Product Code A 3221 and water)

100 mM Ammonium Bicarbonate Solution (prepared from Product Code A 6141 and water)

0.2 mg/ml Proteomics Grade Trypsin Solution (prepared from Product Code T 6567 and 1 mM HCl)

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

The product is a clear, colorless liquid and is supplied ready-to-use. The 8 M Guanidine Hydrochloride Solution may be used as a stock solution to prepare solutions of lower concentrations as appropriate (see Table 1).

Table 1.
Dilution Table - These dilutions are based on a 10 ml starting volume of the 8 M Guanidine-HCl buffered solution.

Desired Molarity	Volume of water to Add to 10 ml of 8 M Guanidine-HCl	Final Volume
8 M	0.0 ml	10.0 ml
7 M	1.4 ml	11.4 ml
6 M	3.3 ml	13.3 ml
5 M	6.0 ml	16.0 ml
4 M	10.0 ml	20.0 ml
3 M	16.7 ml	26.7 ml
2 M	30.0 ml	40.0 ml
1 M	70.0 ml	80.0 ml

Storage/Stability

Store the product at room temperature. The product is stable for at least one year in an unopened container.

Procedure

A. Protein Denaturation and Solubilization of Cell Paste

1. Add 1 ml of the 8 M Guanidine Hydrochloride Solution for every 0.1 gram of wet cell paste.
2. Vortex the suspension for 2 minutes.
3. Centrifuge the suspension at 15,000 x *g* for 10 minutes at room temperature to remove cell debris.
4. After centrifugation, carefully remove the supernatant containing the soluble proteins. Note: For SDS-PAGE analysis, the denaturant must be removed by dialysis or protein precipitation with trichloroacetic acid (TCA, Product Code PROT-PR).

B. Reduction and Alkylation of Extracted Proteins and Tryptic Digestion (Optional)

1. Reduce the protein sample (section A, step 4) by adding 25 µl of the 200 mM Tributylphosphine Solution per 1 ml of protein solution. Incubate the sample at room temperature for 15-30 minutes.
2. Alkylate the protein solution by adding 30 µl of the 0.5 M Iodoacetamide Solution per 1 ml of protein solution. Incubate the sample at room temperature for 1 hour.
3. Quench the excess iodoacetamide with additional TBP. Add 25 µl of the 200 mM Tributylphosphine Solution per 1 ml of protein solution and incubate for 15 minutes at room temperature.
4. Centrifuge the sample at 20,000 *x g* for five minutes at room temperature to pellet any insoluble material.
5. Dialyze the sample against 1 liter of 100 mM Ammonium Bicarbonate Solution for 1 hour at room temperature.
6. Replace the dialysis buffer with fresh 100 mM Ammonium Bicarbonate Solution and continue to dialyze for another 2 hours. If a precipitate forms during dialysis, resuspend the precipitated proteins by pipetting the solution up and down. Transfer the solution to a clean tube. The precipitate will clear upon enzymatic digestion.
7. Add 100 µl of the 0.2 mg/ml Proteomics Grade Trypsin Solution to the dialyzed sample. (For more information, see the Technical Bulletin for Product Code T 6567.)
8. Incubate the sample overnight at 37 °C in a water bath.
9. Peptide solutions can now be analyzed by mass spectrometric methods.

References

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