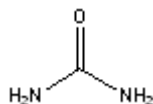


Catalog Number: 103209, 105695, 191450, 194762, 194857, 821519, 821527, 821530, 821531, 821532

Urea

Structure:



Molecular Formula: CH₄N₂O

Molecular Weight: 60.06

CAS # 57-13-6

Synonyms: Carbamide; Carbonyl diamide

Physical Description: White crystalline powder or pellets.

Purity: \geq 99%

Solubility: One gram dissolves in 1 ml water, 10 ml 95% ethanol, 1 ml boiling 95% ethanol, 20 ml absolute ethanol, 6 ml methanol, 2 ml glycerol; Soluble in concentrated hydrochloric acid; almost insoluble in chloroform, ether.¹ Solutions of urea develop a significant concentration of reactive cyanate ions on standing. MP recommends making solutions fresh for each use. Solutions can be treated to remove isocyanic acid. Acidification (100 mM HCl) can be used to drive the equilibrium to favor urea over the isocyanic acid, or a urea solution can be bulk deionized using an ion exchange resin.

Typical procedure for deionizing urea solutions:

1. Dissolve urea in deionized water to the desired concentration.
2. For every 10 ml of solution, add 1 g of Amberlite® IRA-910 (MP # 150323).
3. Stir for one hour at room temperature.

Biuret Reaction: Water solutions decompose on heating, giving off some

NH₃. Pure urea should not give the biuret reaction unless heated above the melting point.¹ Reagent grade urea may give a positive biuret reaction.

Description: Urea is the principal end product of nitrogen metabolism in most mammals, formed by the enzymatic reactions of the Krebs' cycle.

Urea is a mild agent usually used in the solubilization and denaturation of proteins.^{4,8,12,16} It is also useful for renaturing proteins from samples already denatured with 6 M guanidine hydrochloride such as inclusion bodies^{9,17}; and in the extraction of the mitochondrial complex.²⁰ It is commonly used to solubilize and denature proteins for denaturing isoelectric focusing and two-dimensional electrophoresis^{2,4,5,10,16} and in acetic acid-urea PAGE gels.¹⁸ Preparing samples for two-dimensional polyacrylamide electrophoresis (2D-PAGE) involves solubilization, denaturation and reduction in order to completely disrupt the interactions between the proteins.¹⁶ Due to the diversity of samples which are analyzed by 2D gel electrophoresis, there is no single method of sample preparation that works in all cases. Ideally, all non-covalently bound protein complexes and aggregates should be denatured to form a solution of individual polypeptides.⁴ However, regardless of the method used, protein modifications which might result in artifactual spots on 2D maps must be minimized. For example, samples containing urea must not be heated as this may introduce considerable charge heterogeneity due to carbamylation of the proteins. Samples should be kept cold at all times and handling should be kept to a minimum.²¹

Urea in solution is in equilibrium with ammonium cyanate. The form that reacts with protein amino groups is isocyanic acid. Urea in the presence of heat and protein leads to carbamylation of the proteins. Carbamylation by isocyanic acid interferes with protein characterization because isocyanic acid reacts with the amino terminus of proteins, preventing N-terminal sequencing. Isocyanic acid also reacts with side chains of lysine and arginine residues resulting in a protein that is unsuitable for many enzymatic digests. In addition, carbamylation often leads to confusing results from peptides having unexpected retention times and masses.⁶

When performing enzymatic protein digests it is important to remove urea first.²¹ Even though some enzymes will tolerate small amounts of urea, the elevated temperature used for most reactions will lead to carbamylation during the course of the digest. The urea can be removed prior to digest by fast reversed phase chromatography, spin columns, or dialysis.

Urea is used in cell or tissue culture media to increase the osmolality.³

Urea has also been used as fertilizer because of the easy availability of nitrogen; in animal feeds; it is reacted with aldehydes to make resins and plastics; condensed with malonic ester to form barbituric acid; used in the paper industry to soften cellulose; used as a diuretic; enhances the action of sulfonamides; an antiseptic.¹

Typical Use: Urea is typically used at a concentration of 8 M for protein denaturation or solubilization. A final concentration of 5 M urea is commonly used in molecular biology for sequencing gels. To prevent carbamylation, do not heat urea containing buffers above 37° C.

Availability:

Catalog Number	Description	Size
103209	Urea, reagent grade	100 g 500 g 1 kg 5 kg
194762	Urea, cell culture reagent	100 g 500 g 1 kg 5 kg
105695	Urea, purity approximately 99%	1 lb 5 lb
821519 821527 821530 821531 821532	Urea, Ultra Pure, purity not less than 99%	1 lb 5 lb 5 kg 25 kg 25 lb
194857	Urea, molecular biology reagent, purity approximately 98%	100 g 500 g 1 kg 5 kg
191450	Urea, ACS Reagent Grade, purity not less than 99%	500 g 1 kg 5 kg

References:

1. *Merck Index*, 12th Ed., No. 10005.

2. Andrews, A.T., *Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications*, 2nd ed., Oxford Science Publications: New York, NY, p. 252 (1988).
3. Green, R.B., et al., "Hyperosmolality inhibits sodium absorption and chloride secretion in mIMCD-K2 cells." *Am. J. Physiol.*, v. **271** (6 Pt 2), F1248-F1254 (1996).
4. Herbert, B., "Advances in protein solubilization for two-dimensional electrophoresis." *Electrophoresis*, v. **20**, 660-663 (1999).
5. Hochstrasser, D.F., et al., "Methods for increasing the resolution of two-dimensional protein electrophoresis." *Anal. Biochem.*, v. **173**, 424-435 (1988).
6. Lippincott, J. and Apostol, I., "Carbamylation of cysteine: a potential artifact in peptide mapping of hemoglobins in the presence of urea." *Anal. Biochem.*, v. **267**, 57-64 (1999).
7. Maniatis, T. and Efstratiadis, A., *Meths. Enzymol.*, v. **65**, 299 (1980).
8. Marston, F.A.O. and Hartley, D.L., "Solubilization of protein aggregates." *Methods Enzymol.*, v. **182**, 264-276 (1990).
9. Mukhopadhyay, A., "Inclusion bodies and purification of proteins in biologically active forms." *Adv. Biochem. Eng. Biotechnol.*, v. **56**, 61-109 (1997).
10. O'Farrell, P.H., "High resolution two-dimensional electrophoresis of proteins." *J. Biol. Chem.*, v. **250**, 4007-4021 (1975).
11. Ogden, R.C. and Adams, D.A., *Meths. Enzymol.*, v. **152**, 66 (1987).
12. Pace, C.N., *Meths. Enzymol.*, v. **131**, 266 (1986).
13. Poulson, R., *The Ribonucleic Acids*, P.R. Stewart and D.S Letham (eds), Springer Berk. & New York, p. 333 (1977).
14. Paulus, A., et al., *Electrophoresis*, v. **11**, 702 (1990).
15. Paulus, A. and Husken, D., *Electrophoresis*, v. **14**, 27 (1993).
16. Rabilloud, T., "Solubilization of proteins for electrophoretic analysis." *Electrophoresis*, v. **17**, 813-829 (1996).
17. Rudolph, R. and Lilie, H., "In vitro folding of inclusion body proteins." *FASEB J.*, v. **10**, 49-56 (1996).
18. Smith, B.J., *Meth. Mol. Biol.*, v. **1**, 63 (1984).
19. Watmough, N.J., et al., *Biochemistry*, v. **30**, 1317 (1991).
20. Wong, S.Y., Hatefi, Y., *Arch. Biochem. Biophys.*, v. **211**, 643 (1981).
21. MP Quality Control.