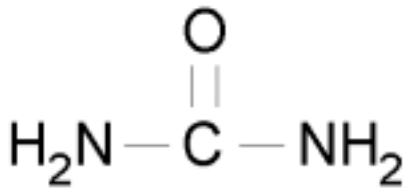
ProductInformation

UREA
Product Numbers:
U6504, Electrophoresis Reagent
U5378, Molecular Biology Reagent
U4128, Cell Culture Tested
U0631, SigmaUltra
U4884, USP
U5128, ACS Reagent
U1250, Pellets

Storage Temperature Room Temperature

Cas #: 57-13-6
Synonyms: Carbamide, Carbonyl diamide

Product Description



Appearance: U4128 and U1250 are white pellets; others are white powder
Molecular Formula: CH_4N_2O
Molecular Weight: 60.06

Urea is a mild chaotropic agent useful for the solubilization and denaturation of proteins.[1-3] It is also useful for renaturing proteins from samples already denatured with 6 M guanidine hydrocholride such as inclusion bodies.[4,5] It is commonly used to solubilize and denature proteins for denaturing isoelectric focusing and two-dimensional electrophoresis.[2,3,6,7,8] Preparing samples for two-dimensional polyacrylamide electrophoresis (2D PAGE) involves solubilization, denaturation and reduction in order to completely disrupt the interactions between the proteins.[2] 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.[3] 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 hetero-geneity due to carbamylation of the proteins (see information below). Samples should kept cold at all times and handling should be kept to a minimum.

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.[9]

When performing enzymatic protein digests it is important to remove urea first. 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.[10] It is the principal end product of nitrogen metabolism in most mammals, formed by the enzymatic reactions of the Kreb's cycle.

Preparation Instructions
One gram of urea dissolves in one ml water, 10 ml 95% alcohol, 20 ml absolute alcohol, 6 ml methanol, or 2 ml glycerol. It is almost insoluble in chloroform and ether, though it is soluble in concentrated HCl. 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 > 37°C**.

Storage/Stability
The solid material should be stored at room temperature. Solutions of urea develop a significant concentration of reactive cyanate ions on standing. **Urea solutions should always be used fresh** and may 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.

Procedure
To deionize urea solutions:
1. Dissolve the urea in deionized water to the desired concentration
2. For every 10 ml of solution, add 1 g of mixed bed resin (Product No. M8032).
3. Stir for one hour at room temperature.
4. If the blue color of the resin persists, filter the resin and the solution is ready to use. If blue color does not persist, add more resin and stir again.

Product Profile
U6504 has been tested and found suitable for use in electrophoresis. U5378 has been found to be free of detectable levels of DNase, RNase and protease. U4128 has been found to be suitable for cell culture applications. U0631 is SigmaUltra grade, having been tested to meet low metal content specifications. U4884 meets the USP specifications and U5128 meets the ACS specifications.

References

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AC 9/29/00