Solubilization

Protocol

Bulletin 6220

Sample Solubilization and Preparation Methods

Protein solubilization is sample dependent. Several solubilization solutions and protocols are detailed in this section.

Standard Sample Solubilization Solution

The sample solubilization solution described in Table 1 is commonly used as a general extraction solution and provides a good starting point for sample preparation. This solution is also used for IPG strip rehydration, for sample application by in-gel rehydration or cup loading, and for sequential extraction of more complex samples. It is available from Bio-Rad as reagent 2 in the ReadyPrep™ sequential extraction kit. A similar IPG rehydration/sample buffer ideal for *E. coli* samples can be ordered as ReadyPrep 2-D starter kit rehydration/sample buffer. Each vial in the starter kit reconstitutes to 10 ml of 8 M urea, 2% CHAPS, 40 mM DTT, 0.2% Bio-Lyte® 3/10 ampholyte.

Table 1. Sample solubilization solution (ReadyPrep reagent 2).

Components	Amount
8 M Urea	47 ml of 8.5 M stock (Table 9.2) or 24 g dry urea dissolved in 25 ml of H_2O
50 mM DTT or 2 mM TBP	385 mg or 500 µl of 200 mM TBP stock
4% CHAPS	2 g
0.2% Carrier ampholytes	See Table 9.4
0.0002% Bromophenol Blue*	100 µl of 0.1% stock
Water	Adjust to 50 ml

* Bromophenol Blue is included in trace amounts in rehydration solutions both to view the rehydration of the strip with the solution and to observe the early stages of electrophoresis. It is not required for solubilization.

Dissolve the urea in about 25 ml of water with stirring. Add the CHAPS, ampholytes, and Bromophenol Blue and adjust the solution to a final volume of 50 ml. Tris may be added at 10–40 mM if pH control is important. Tris will increase the conductivity and extend the time required to focus the IPG strips. Add DTT or TBP immediately before use. Use carrier ampholytes that span the pH range of the IPG strip according to Table 1. Urea stock solutions should be used soon after they are made, or treated with a mixed-bed ion exchange resin to avoid protein carbamylation by cyanate, which forms in old urea. Table 2 and the section on urea stock solutions describe the preparation and storage of urea stock solutions. Store sample solubilization solution in aliquots at –20°C. Thaw only the required number of aliquots and discard leftover solution. Add sample solution to the protein sample so the final concentration of urea is ≥6.5 M. Solid urea may be added as necessary. Proteins may be directly extracted in sample solubilization solution using at least 9 ml of solution for each 1 ml of packed cell pellet. Use sample solubilization solution to rehydrate IPG strips.

Table 2. Urea stock solution, 8.5 M.

Urea Stock Solution Components	Amount
8.5 M Urea	510 g
Water	Adjust to 1 L

Dissolve the urea in about 600 ml of water with gentle heating (<30°C) and vigorous stirring with a heavy stirbar. Remove from the heat source as soon as the urea dissolves. Add 5 g of Bio-Rad deionizing resin (Bio-Rex® 501-X8) and stir for 10 min. If the resin de-colors, add an additional 5 g and repeat until the resin no longer loses color. Filter the solution through Whatman No.1 paper using a Buchner funnel.

Store convenient aliquots of this urea solution at –20°C until required. This deionized 8.5 M stock can be used to make up all urea-containing solutions. Do not store urea solutions at room temperature (or 4°C) any longer than necessary. Urea in solution exists in equilibrium with ammonium cyanate, which can cause irreversible protein modification and interfere with IEF.

Urea Stock Solutions

Urea is a chaotropic agent comonly used in IEF sample preparation. To prepare an 8.5 M urea stock solution, see Table 2. High-purity urea should be used for IEF. Charged species can be removed by addition of a mixed-bed ion exchange resin. The resin is then removed by filtration. Urea should not be heated above 30°C because carbamylation of the sample may occur, which gives rise to charged artifacts detected in the second-dimension gel.

For some applications, it is convenient to prepare a saturated urea solution (9.8 M) containing 4% CHAPS (Table 3). By diluting samples with the 9.8 M urea solution, the final urea and CHAPS concentrations remain high even when large volumes of aqueous protein sample must be denatured. The solution should be stored frozen in aliquots. Thaw enough for use when needed. Add reducing agent and ampholytes immediately before use. Discard unused reagent.

Table 3. Urea/CHAPS stock solution.

Components	Amount
9.8 M Urea	29.4 g
4% CHAPS	2 g
Water	Adjust to 50 ml

Dissolve the urea in about 25 ml of water with stirring. Add the CHAPS and adjust the final volume to 50 ml. Store in aliquots at –20°C.

This is an excerpt from Bio-Rad's comprehensive manual, 2-D Electrophoresis for Proteomics (Bulletin 2651).

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