Lysis and Protein Extraction from \( ^{32}\text{P}-\text{Labeled} \) B Cells with TriPure Isolation Reagent
AfCS Procedure Protocol PP00000154
Version 1, 05/12/03

The following protocol is used to prepare protein extracts from \( ^{32}\text{P}-\text{labeled} \) B cells. This preparation method provides total cellular protein samples that are free of contaminating nucleic acids. The protein extracts are used for analysis of \( ^{32}\text{P}-\text{labeled} \) proteins by two-dimensional gel electrophoresis.

**Caution:** when working with TriPure reagent, use gloves and eye protection. Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

The procedure is carried out at room temperature except where indicated.

**Procedure**

1. Following \( ^{32}\text{P}-\text{labeling} \) and stimulation, transfer cell suspension containing \( 8 \times 10^7 \) B cells to a capped 2-ml microcentrifuge tube. Harvest the cells by centrifugation for 10 sec in a variable-speed microcentrifuge set at 3,000 x g. Carefully remove the supernatant solution with a P1000 pipette or aspirator needle and discard in radioactive waste. Be careful not to disturb the cell pellet.

2. Add 1.5 ml of TriPure reagent and lyse cells thoroughly by repetitive pipetting with a P1000 pipette. The cell pellets may not always dissolve completely. Pieces of pellet may remain even after extensive pipetting and tend to plug the pipette tip.

3. Centrifuge the samples at 12,000 x g for 10 min at 4 °C using a microcentrifuge in a cold room.

4. Transfer the supernatant fractions to a fresh 2-ml microcentrifuge tube, avoiding contact with the pellet.

5. Incubate the samples for 5 min at room temperature to permit the complete dissociation of nucleoprotein complexes.

6. Add 0.3 ml of chloroform and cap sample tubes securely.

7. Shake tubes vigorously by hand for 15 sec, and incubate at room temperature for 10 min. Make sure the chloroform has completely mixed with the aqueous solution to form a homogeneous mixture.

8. Centrifuge the samples at 12,000 x g for 15 min at 4 °C. Following centrifugation, the mixture separates into a lower red (phenol-chloroform) phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TriPure reagent used for homogenization.

9. Carefully remove ALL of the aqueous (upper) phase with a P1000 pipette or needle aspirator. Discard in radioactive waste. Some of the cloudy interphase can be removed to ensure all of the aqueous phase is removed. It may be helpful to tilt the tubes in order to better visualize the interphase between the aqueous and organic phases.

10. Add 0.45 ml of 100% ethanol to precipitate the DNA from the interphase and organic phase. Cap tubes and mix samples thoroughly by vigorously inverting the tubes several times.
11. Incubate the tubes at room temperature for 3 min.
12. Sediment the precipitated DNA by centrifugation at 5,000 x g for 5 min at 4 °C. The tubes should be placed in the microcentrifuge with the cap hinges facing outward to mark the side of the tube where DNA will be pelleted.
13. Carefully transfer phenol-ethanol supernatant to a new tube, making sure to avoid contact with the DNA pellet aligned with the cap hinge.
14. Estimate the volume and pipette half of each sample into a new tube. Sample must be split into two tubes to allow sufficient room to add isopropanol.
15. Precipitate the protein from the phenol-ethanol supernatant by adding 1.15 ml of isopropanol to each tube. Vortex the tubes for 10 sec.
16. Incubate the tubes for 10 min at room temperature and sediment the protein precipitate by centrifugation at 12,000 x g for 10 min at 4 °C.
17. Remove the supernatant fraction and discard in radioactive waste. Add 1.8 ml of guanidine hydrochloride in 95% ethanol (guanidine HCl in 95% ethanol) to each tube. Vortex briefly to loosen the pellet from the side of the tube, and incubate for 20 min at room temperature. The protein pellet can be stored for several nights at 4 °C in the guanidine HCl in 95% ethanol solution.
18. Collect the protein pellet by centrifugation at 7,500 x g for 5 min at 4 °C. Note: the protein pellet does not dissolve during this washing step.
19. Remove supernatant and repeat steps 17 and 18 two additional times.
20. Add 1.8 ml of 95% ethanol and vortex to loosen protein pellet. Incubate for 20 min at room temperature.
21. Collect the protein pellet by centrifugation for 5 min at 12,000 x g at 4 °C. The protein precipitate in 95% ethanol can be stored overnight at 4 °C.
22. Remove supernatant (ethanol) and discard in radioactive waste. Dry the pellet in a Speed-Vac concentrator for 10 min without heating.
23. Add 125 µl of 2-D rehydration buffer 1 (2-D RB1). Vortex tubes for 60 sec.
24. Incubate tubes at room temperature for 45 min and vortex occasionally.
25. Centrifuge tubes at 12,000 x g in a microcentrifuge for 2 min to pellet any remaining debris. The protein pellet should dissolve completely in the buffer.
26. Carefully remove the supernatant fraction, avoiding contact with the debris pellet. Combine the supernatants from the two tubes for each sample into a single tube and vortex briefly.
27. The protein preparations can be used directly for protein determination and applied to first-dimension isoelectric focusing. Alternatively, the samples can be stored at -20 °C overnight or at -80 °C for several days.
Reagents and Materials
TriPure reagent: Roche Applied Science; catalog no. 1-667-157

Chloroform: Sigma-Aldrich; catalog no. C5312

Ethanol, 100%: Sigma Aldrich; catalog no. E702-3

Isopropanol: Sigma-Aldrich; catalog no. I0398

Guanidine hydrochloride in 95% ethanol (guanidine HCl in 95% ethanol); AfCS Solution Protocol PS00000509

Ethanol, 95%: Sigma-Aldrich; catalog no. E714-8

Speed-Vac concentrator; Savant Instruments; model no. SC110-120

2-D Rehydration buffer 1 (2-D RB1); AfCS Solution Protocol PS00000481

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