

SOP:SP019

Removal of SDS by Paired-ion Extraction Protocol

Materials and Reagents:

1. Acetone
2. Glacial acetic acid
3. Triethylamine (note 1)
4. Burdick and Jackson H₂O
5. Lyophilized sample in 35 ml centrifuge tube(s)
6. 35 ml centrifuge tubes
7. SS34 rotor
8. High speed centrifuge
9. 150 ml flask with screw-top lid
10. Stirbar
11. Stirplate
12. Air bath
13. -20°C freezer
14. Glass pipets (5 and 10 ml)
15. Graduated cylinder (100 ml)

Protocol:

1. ____ In fume hood, add 85 ml of acetone, 5 ml of glacial acetic acid, 5 ml of triethylamine, 5 ml water to a 150 ml flask containing a stir bar (note 2).
2. ____ Place cap on flask and stir on stir plate for 2 minutes to thoroughly mix the solvents.
3. ____ Pipet solvent to each tube containing lyophilized sample (note 3).
4. ____ Cap centrifuge tube(s) and vortex vigorously to disperse the sample in the solvent.
5. ____ Place at -20°C for 4 to 16 hours.
6. ____ Centrifuge at 27,000 x g (15,000 rpm), 4°C, 30 min.
7. ____ Pour off supernatant, making certain not to lose the pellet (notes 4 and 5).
8. ____ Repeat steps 1-7.
9. ____ Add 30-35 ml of ice-cold acetone to the pellet(s) from step 8.
10. ____ Vortex vigorously to disperse pellet(s) in the acetone.
11. ____ Place at -20°C for at least four hours.
12. ____ Centrifuge 27,000x g, 4°C, 30 min..
13. ____ Pour off supernatant. This should remove residual triethylamine.
14. ____ Place tube with pellet in air bath and pass a gentle stream of N₂ over the pellet to volatize residual acetone (note 6).
15. ____ Add the buffer of your choice (usually 10 mM ammonium bicarbonate) to the pellet.
16. ____ Sonicate, or gently vortex to suspend the pellet.

16. _____ Perform BCA assay and SDS-PAGE on the extracted sample.
17. _____ Aliquot resuspended pellets for long term storage in cryovials suitable for -80°C freezer (note 7).

QC Procedures:

1. SDS-PAGE
2. SDS concentration determination

Notes:

1. The triethylamine used needs to be fresh.
2. Only use glass pipets.
3. A general rule of thumb is to add 1 ml of extraction solvent for every 1 ml of sample that was lyophilized. If your sample is not completely dry, the final concentration of water cannot be greater than 5%.
4. It is okay to leave some solvent in the tube at this point. The important thing is to not lose your sample pellet.
5. Discard of all solvents containing acetone as hazardous waste.
6. See SOP SP031 for use of the N_2 Bath.
7. Default quantity shipped by the TB Contract is 1.0 mg.

References:

William H. Konigsberg and Lou Henderson. 1983. Removal of Sodium Dodecyl Sulfate from Proteins by Ion-Pair Extraction. *Methods in Enzymology*. 91: 254-258.