Wessel & Fluegge (1) protein precipitation

Material:

- 1.5 ml microtubes (chloroform-resistant ➔ PE or PP) / 15 ml Falcon
- Methanol p.A. or better
- Chloroform p.A. or better
- H20 bidest.
- Centrifuge equipped with a rotor for microtubes/Falcons (9000 g needed).

Method:

1. To 100 µl /1 ml sample in a microtube/Falcon add 400 µl/4 ml methanol, mix thoroughly and centrifuge for 10 s at 9000 g.
2. Add 100 µl / 1 ml chloroform, mix and centrifuge for 10 s at 9000g. For samples containing a high amount of lipids (e.g. liposomes) use 200 µl /2 ml of chloroform instead.
3. Add 300 µl / 3 ml H2O bidest, mix thoroughly and centrifuge at 9000 g for 1 min to separate phases. Three phases will become visible. An upper H2O-methanol phase, a protein interphase and a lower chloroform phase.
4. Remove the upper phase carefully without disturbing or touching the protein interphase.
5. Add another 300 µl / 3 ml of methanol to the remaining phases, mix thoroughly and centrifuge for 2 min at 9000 g. This pellets the protein.
6. Remove the supernatant carefully and dry the pellet (Until no chloroform smell is detectable anymore.).
7. The dried pellet can now be solubilized in an appropriate buffer for further processing e.g., Laemmli buffer for SDS-PAGE separation or 8 M Urea buffer for in-solution protein digestion.

References:

1. Wessel, D, and Flügge, UI A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Analytical Biochemistry* **138**, 141 143