Methanol Precipitation of Proteins

Tobias von der Haar[1]

[1]University of Kent at Canterbury

Sep 04, 2019

1 Works for me

dx.doi.org/10.17504/protocols.io.icecate

Tobias von der Haar

ABSTRACT

This protocol is adapted from Wessel and Flugge, 1984. It is suitable for precipitating very dilute proteins, and proteins from solutions containing detergents.

1 Select a suitable container for the precipitation. This protocol requires the addition of large amounts of additional liquid to the original sample, so the container needs to be sufficiently large as well as suitable for centrifugation.

Maximal sample sizes for the following container volumes are (tube volume: maximal sample volume): 1.5 ml: 0.16 ml; 2 ml: 0.22 ml; 10 ml: 1.1 ml; 15 ml: 1.6 ml; 50 ml 5.5 ml.

2 Place your sample in the selected container. Note the sample volume, and use this to calculate the amount of reagents to add in subsequent steps.

3 Mix the sample with 4 volumes of methanol. Vortex, then add 1 volume of chloroform and vortex again. Add 3 volumes of water, vortex, and centrifuge at 9000 g, 4°C for five minutes.

4 Followig the centrifugation you should observe two phases, an upper aqueous phase and a lower solvent phase. The protein is located at the interface between the phases (it may be visible as a white film, or it may not be visible at all if the protein amount is small).

Pipet away and discard the upper, aqueous phase, taking care not to disturb the interface (it is ok to leave some of the aqueous phase behind).

5 Add 3 volumes of methanol to the tube, vortex, and centrifuge at 9000 g, 4°C for ten minutes.

6 Pour off the liquid and dry the pellet by standing the tube, open, upside down on a clean, dry tissue. Dissolve the pellet in an appropriate volume of buffer (eg SDS sample buffer if the precipitate is to be analyses by SDS PAGE).

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