Protocol for chloroform-methanol precipitation

1. 4 parts of methanol (0.4 mL) are added to 1 part (0.1 mL) of protein sample and vortex.
2. Centrifuge the mixture 10s at 9000g.
3. Add 2 parts (0.2 mL) of chloroform and vortex.
4. Centrifuge the mixture 10s at 9000g.
5. For phase separation, add 3 parts (0.3 mL) of water and vortex hard.
6. Centrifuge at 10000g for 15 min at room temp. Ensure interphase is formed.
7. Remove the top aqueous layer and discard, leaving the white interphase layer containing the protein. Add same volume (1:1) of 100% methanol to the lower chloroform base, vortex hard.
8. Centrifuge (5 min 10000g at room temp) and ensure the opaque protein pellet is visible at the bottom of the tube. Remove all supernatant, ensuring the pellet remains at the bottom of the tube. Repeat steps 7 & 8 twice to complete the methanol wash.
9. Remove the supernatant and dry the protein by speed vacuum (~5 min).