Minute™ High Efficiency Protein Precipitation kit

Cat No. WA-006

Description

Protein precipitation stands out as a clear strategy for concentrating proteins and eliminating interfering substances present in protein samples, such as salts, lipids, and other elements that might disrupt subsequent applications. Among the widely employed techniques, trichloroacetic acid (TCA) precipitation emerges as a straightforward and efficient method. However, proteins precipitated through the TCA method typically undergo denaturation, often resulting in reduced solubility.

A notable drawback of the conventional TCA/acetone precipitation approach is its diminished efficacy when dealing with samples featuring low protein concentrations. Higher protein concentration samples are precipitated much more efficiently compared to those with lower protein concentrations. In response to these limitations, we have innovated a high-efficiency protein precipitation kit, a refinement of the traditional TCA method. This kit is characterized by its simplicity, rapidity, and heightened effectiveness. It enables the effective precipitation and concentration of proteins at low concentrations in less than 30 minutes.

Key attributes: Streamlined, swift, and notably user-friendly compared to comparable products. Every step can be executed at room temperature, enhancing convenience. Particularly advantageous for precipitating protein samples characterized by low concentrations.

Components:	Protein precipitation Solution	30 ml
	Solution P	6 ml
	Washing Solution	30 ml

Shipping and storage: The product is shipped at ambient temperature and stored at RT.

Protocol (read the entire protocol prior to use)

1. Move the protein sample solution into a test tube, such as a 1.5 ml or 2.0 ml microfuge tube. Ensure that the volume does not exceed 0.7 ml for 1.5 ml tubes or 1.0 ml for 2.0 ml tubes. While larger test tubes are an option, keep in mind that a larger centrifuge may be necessary for processing.
2. Add an equal volume of protein precipitation solution to the protein sample (e.g., if the sample volume is 0.5 ml, add 0.5 ml of protein precipitation solution to the tube), followed by solution P. The quantity of solution P added should be 1/10 of the total volume (for example, if the combined volume of protein solution and protein precipitation solution is 1.0 ml, add 100 µl of solution P). Thoroughly mix the contents by vortexing for 10 to 20 seconds, and then incubate at room temperature for 5 to 10 minutes (Alternatively, it can be placed on ice if preferred).
   Note: In cases where downstream experiments involve Mass Spectrometry, refrain from adding solution P to prevent potential interferences.
3. Centrifuge in a microcentrifuge at top speed (about 14,000-16,000 X g) for 10 min. Pour the supernatant completely and add 0.5 ml (assume the starting protein sample is 0.5 ml) of washing solution to the tube. Invert the tube a few times.

4. Centrifuge the sample in a microcentrifuge at maximum speed for 5 minutes. Carefully discard the supernatant and place the tube in an invert position for a few minutes. If necessary, perform an additional washing step. Resuspend the pellet in a buffer containing detergent, such as 0.5% SDS for SDS-PAGE or 2D gel rehydration buffer. Determine the protein concentration using the BCA kit from Pierce.
   Note: The precipitated proteins could be denatured with lost biological activities.

Tech Notes

Following the addition of solution P to the precipitation mix, the appearance of a white precipitate is a normal occurrence. The protein pellet formed during precipitation must be dissolved in a buffer containing detergent for subsequent analysis. If the pellet proves challenging to dissolve, consider trying the following approaches:

1. Re-suspend the pellet, adjusting the volume to 50-100 µl based on pellet size, in PBS containing 0.8% SDS and 20 mM DTT by pipetting up and down. Boil the sample for 2-3 minutes.
2. Centrifuge at 10,000 X g for 2-3 minutes and collect the supernatant for analysis. The majority of the protein should be dissolved, although a residual white pellet may still be visible post-centrifugation. This is primarily attributed to non-protein precipitation carriers in solution P.
3. Alternatively, resuspend the pellet in 50-100 µl of 1X SDS-PAGE loading buffer, heat the solution, and centrifuge at 10,000 X g. Collect the supernatant for analysis.