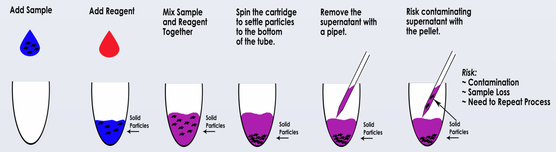
Protein Precipitation and Separation Without Pipetting

Rolf Schlake

Applied Separations, Inc., 930 Hamilton Street , Allentown, PA 18101 www.appliedseparations.com

Abstract

Salting out, adding a solvent, altering the pH are various means to precipitate proteins. In a subsequent two-step process, the mixture is then centrifuged and the supernatant is pipetted off to separate it from the precipitate. Sometimes there is no distinct separation layer making it difficult for technicians to draw up a clean sample. Additionally, the process is not easily automatable. The traditional process is shown in the diagram below.



Traditional Method

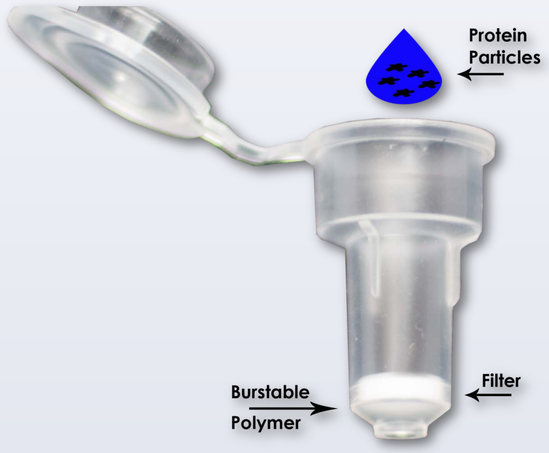
Introduction

A new Burst device has been produced to eliminate the pipetting step, thereby eliminating the possible reintroduction of the precipitate into the supernatant. The device also gives the ability to automate the procedure. (See Steps 1 - 3.)

The device is applicable to many other applications where transfer by pipette is necessary. This paper will show the construction of this unique device and describe its use in protein precipitation using trichloroacetic acid (TCA).

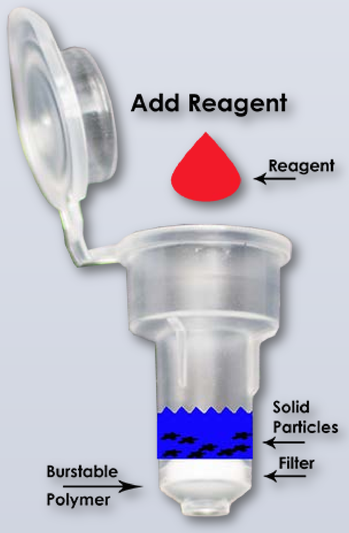
Step 1

Add 500µL Protein Sample



Step 2

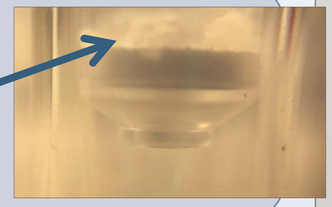
Add 100µL Trichloracetic Acid (TCA)



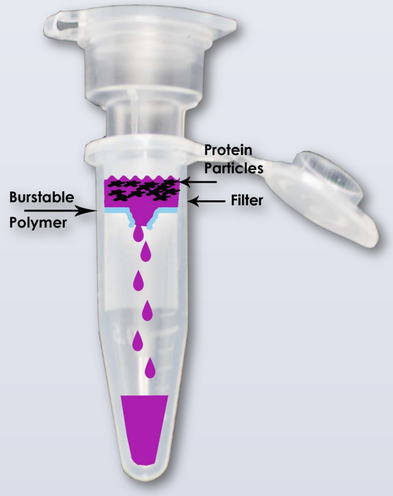
Step 3

Liquid passes through the filter and is stopped by the burstable polymer.

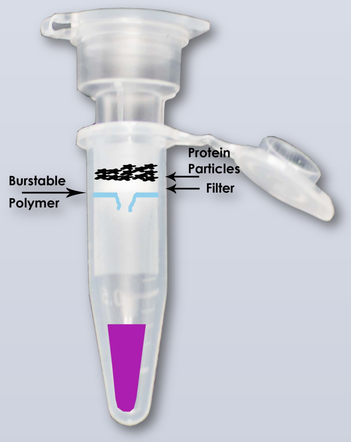
The reaction happens above the filter.

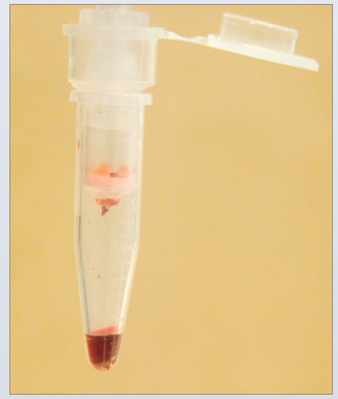


Centrifuge to break the polymer.



Wash the pellet 3x with 200µL Acetone





Conclusion

- The “Burst” Technique eliminates the problem of pipetting to remove the supernatant and to wash the protein pellet with acetone
- Sample loss is minimized
- Protein precipitation for SDS-PAGE was more rapid than the traditional method.