ReadiUse™ TCA Deproteinization Test Sample Preparation Kit

Ordering Information	*Storage Conditions*
Cat#: 19501 (200 Assays)	Refrigerated

Introduction

The analysis of amino acids, ions, metabolites and other small molecules is frequently hindered by the interference of lipids, protein and enzymes present in biofluids, cell and tissue lysates. Therefore, deproteinization is a necessary step in many procedures prior to analysis of biological samples. AAT Bioquest's Deproteinization Sample Preparation Kit offers a rapid method for removing proteins in biological samples. Proteins are precipitated by applying trichloroacetic acid (TCA). After removal of precipitated proteins, the pH of the sample is neutralized with neutralization solution. TCA based deproteinization has been successfully and widely used in sample preparation prior to quantitation of small molecules, such as glycogen, ATP, cAMP, glutathione and antioxidants. The TCA method provided in this kit can also be used for removing protein in large scale. Biological samples prepared using this kit can be directly used in various biochemical analysis.

Kit Components

Components	**Amount**
Component A: TCA	1 bottle (2 mL)
Component B: Neutralization Solution	1 bottle (2 mL)

Assay Protocol

Note: Place kit components on ice before use. Store the kit at room temperature.

1. Protein Precipitation:

1.1 Prepare a clear solution sample with protein concentration less than 50 mg/mL after homogenization and centrifugation. Keep protein samples on ice.
Note: High protein concentration samples might need to be diluted.

1.2 Add 10 µL of cold TCA (Component A) into 100 µL of protein sample and vortex briefly to mix well.

1.3 Keep the sample on ice for 10 minutes. Centrifuge at 12,000 rpm for 5 minutes. Transfer supernatant (deproteinized sample) to another tube.
Note: It is recommended to neutralize samples in TCA (Step 2.1) and use for relevant assays immediately. However, deproteinized samples in TCA may be stored at -70ºC for up to one month.

2. TCA Neutralization:

2.1 To neutralize excess TCA, add 10 µL of cold Neutralization Solution (Component B) into the collected supernatant and vortex briefly to mix well.
2.2 Keep the samples on ice for 5 minutes. Samples are now deproteinized and neutralized.
2.3 (Optional) Read absorbance at 280 nm (OD) to check if the samples are deproteinized.

Data Analysis

The absorbance OD_{280nm} in blank samples (buffer only without protein) is used as a control, and is subtracted from the OD of protein samples.

Note: Addition of TCA and Neutralization Solution (total volume about 120 µL) dilutes protein concentration in original samples (initial sample volume 100 µL). Dilution factor can be calculated by the following formula:

$$\%\ original\ protein\ concentration = \frac{initial\ sample\ volume}{(initial\ sample\ volume + TCA\ volume + Neutralization\ Solution\ volume)}$$

For example: 100 µL /120 µL= 83%

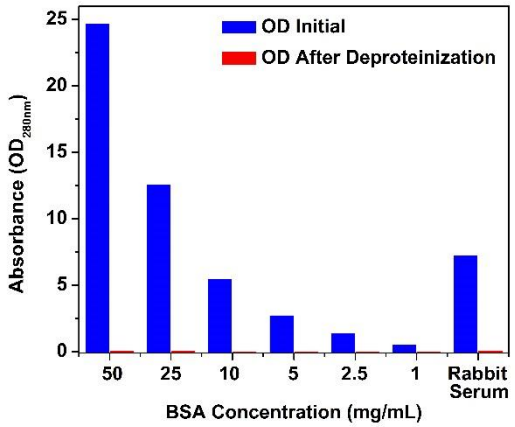

Figure 1. TCA-based deproteinization of protein samples. Bovine serum albumin (BSA) samples with protein concentration less than 50 mg/mL and a rabbit serum sample at the concentration about 14.2 mg/mL were deproteinized using Deproteinization Sample Preparation Kit (Cat#19501). More than 98% of protein in all samples was removed with TCA method.

References

1. Hunter, G (1956) A method for deproteinization of blood and other body fluids. J Clin Path 10: pp. 161-164
2. Ralston, PB; Strein, TG (1997) A study of deproteinization methods for subsequent serum analysis with capillary electrophoresis. Microchem J 55(2): pp. 270-283
3. Gips, CH; Wibbens-Alberts, M (1968) Ammonia determination in blood using the tca direct method. Clin Chim Acta 22(2): pp. 183-186