

## Deproteinization Sample Preparation Kit

### Introduction

Biological fluids, cell lysates, and tissue lysates often contain high levels of lipids, proteins, and enzymes, which can interfere with the detection of small molecule substances such as amino acids, inorganic ions, and various metabolites. Therefore, protein removal is an essential step in biological sample processing prior to detection.

This product is based on the principle of protein precipitation using trichloroacetic acid (TCA), enabling rapid removal of proteins from biological samples. The operation procedure consists of two steps: first, add TCA to precipitate proteins in the sample and remove the precipitate; then, add a neutralization solution to the supernatant to adjust the sample pH to neutral. The treated supernatant can be directly used for subsequent detection.

This product is suitable for sample pretreatment in the quantitative detection of small molecule substances such as glycogen, adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), glutathione, and various antioxidants. It features established methodology and stable results. This kit is suitable for protein removal operations in both small-scale and large-scale sample processing, and the treated biological samples can be directly used in various biochemical detection assays.

### Components and Storage

Components	200 T	Storage
TCA	2 mL	4°C away from light
Neutralization Solution	2 mL	4°C
Shipping: Blue ice		Shelf life: 12 months

### Protocol

#### 1. Protein precipitation

- a. Sample preparation: Centrifuge the homogenized sample and collect the supernatant as the protein sample. Ensure the protein concentration is below 50 mg/mL. Keep the sample on ice throughout the procedure.

**\*Note:** If the protein concentration is high, it is recommended to dilute appropriately.

- b. Add TCA precipitate protein: Take 100  $\mu$ L of the above protein sample, add 10  $\mu$ L of pre-chilled TCA, and

vortex briefly to mix well.

- c. Incubation and centrifugation: Keep the mixture on ice for 10 minutes, then centrifuge at 12,000 rpm for 5 minutes. After centrifugation, carefully transfer the supernatant (i.e., the deproteinized sample) to a new centrifuge tube.

**\*Note:** It is recommended to neutralize the deproteinized sample as soon as possible and use it for subsequent assays. If not used immediately, the sample can be stored at -70°C for up to one month.

## 2. TCA neutralization

- a. Neutralizing residual TCA: Add 10 µL of pre-chilled Neutralization Solution to the collected supernatant (from step 1.c) and vortex briefly to mix well.
- b. Neutralization incubation: Keep the mixture on ice for 5 minutes. The sample is now deproteinized and neutralized.
- c. Effectiveness verification (Optional): Measure the absorbance of the sample at 280 nm (OD280) to assess whether proteins have been effectively removed.

## 3. Example data analysis and figures

% original protein concentration =  $(\text{initial sample volume}) / (\text{initial sample volume} + \text{TCA volume} + \text{Neutralization solution volume})$

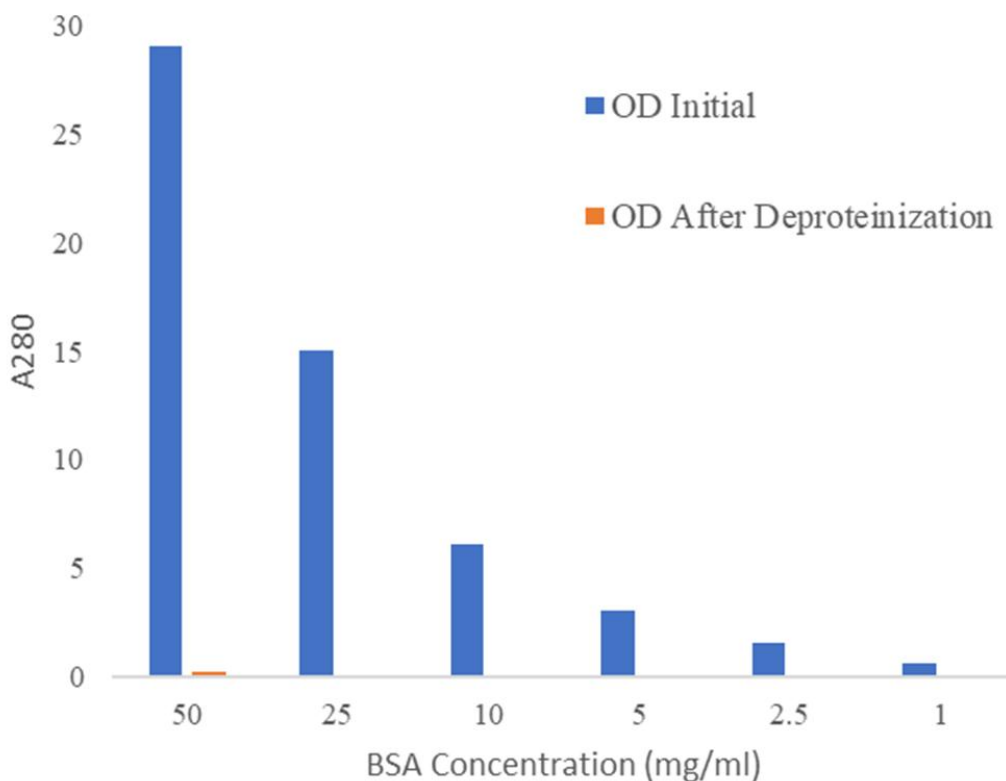
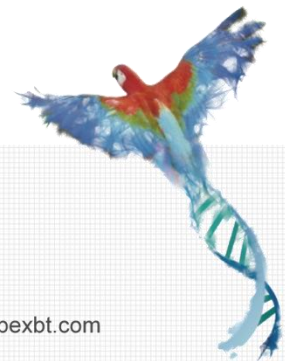
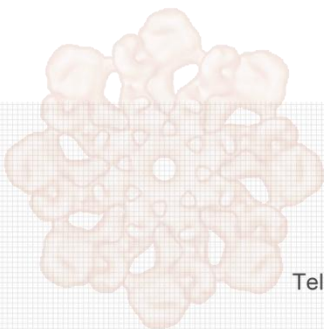


Figure 1. TCA-based deproteinization of protein samples. Bovine serum albumin (BSA) samples with protein concentration less than 50 mg/mL was deproteinized using TCA Deproteinization Test Sample Preparation Kit (Cat#K4064). More than 99% of protein in all samples was removed with TCA method.

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## ■ Note

1. This product is for scientific research use only.



**APEx BIO Technology**

**[www.apexbt.com](http://www.apexbt.com)**

7505 Fannin street, Suite 410, Houston, TX 77054.

Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: [info@apexbt.com](mailto:info@apexbt.com)