

# Deproteinizing Sample Preparation Kit II (TCA method)

2/15

(Catalog # K823-200; 200 assays; Store at room temperature)

## I. Introduction:

The study of biofluids, cell, bacteria, yeast, and tissue lysates has delivered vital information regarding their biochemical composition. These biological samples comprise a rich, complex list of lipids, proteins, small organic molecules, ions, and, depending on the sample, exogenous molecules (i.e. drugs). Several small organic molecules can also be found free and/or bound to other macromolecules. These macromolecules (lipoproteins, proteins, enzymes) interfere or degrade low molecular-weight metabolites. Therefore, sample deproteinization is often required. Chemical deproteinization procedure such as Trichloroacetic acid (TCA) is one of the most utilized methods to accomplish sample deproteinization. BioVision's Deproteinizing Sample Preparation Kit II offers an excellent alternative when metabolite lability affects the accurate detection and estimation of small molecule concentrations and when organic solvents or ultrafiltration cannot be used. TCA precipitates proteins by lowering the sample pH drastically. After removal of precipitated proteins, the pH of the sample is neutralized with neutralization buffer that is provided in the kit. The kit is easy to follow, convenient, and can be utilized for the preparation of a large number of samples in parallel. Samples prepared using this kit can be directly used in various bioassays.

## II. Applications:

- Estimation of low molecular weight metabolites in biofluids
- Estimation of low molecular weight metabolites in cell/tissue lysates

## III. Sample Type:

- Biofluids: serum, plasma, whole blood, saliva, urine etc.
- Lysates: cell culture, mammalian tissue, yeast, bacteria etc.

## IV. Kit Contents:

| Components              | K823-200 | Cap Code | Part Number |
|-------------------------|----------|----------|-------------|
| TCA                     | 3 ml     | NM       | K823-200-1  |
| Neutralization Solution | 4 ml     | NM       | K823-200-2  |

## V. Storage Conditions and Reagent Preparation:

Store kit at room temperature. Place kit components on ice to chill before use.

- **Neutralization Solution:** If precipitation is observed, shake bottle a few times to resuspend precipitate.

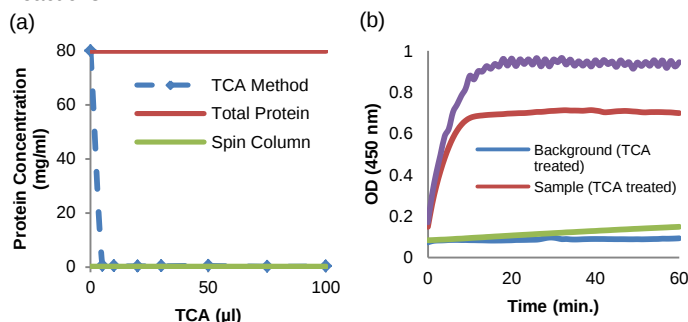
## VI. Deproteinizing Sample Preparation Protocol:

The following protocol can be proportionally scaled for preparation of larger or smaller sample volumes.

- 1. Protein Precipitation.** For samples with high protein concentration (e.g. serum), mix 100  $\mu$ l sample with 15  $\mu$ l of cold TCA and for samples with protein concentration less than ~ 25 mg/ml (e.g. tissue lysate, cell lysate, and yeast lysate), mix 150  $\mu$ l sample with 15  $\mu$ l of cold TCA in a 1.5 ml microcentrifuge tube. Keep the sample on ice for 15 min. Centrifuge at 12,000  $\times$  g for 5 min. Carefully transfer supernatant (~ 100  $\mu$ l for high and ~ 150  $\mu$ l for low protein concentration respectively) to another tube. Samples are now deproteinized,
- 2. Sample Neutralization:** To neutralize excess TCA, add 10  $\mu$ l of cold Neutralization Solution to the collected supernatant. Mix well. Vent sample tube as there may be formation of CO<sub>2</sub>. Place sample on ice for 5 min. Samples are now deproteinized, and neutralized and can be directly used in a variety of assays.

### Notes:

- a. Analysis of samples is recommended to be carried out as soon as samples are deproteinized. However, low pH (deproteinized) samples may be stored at -70°C for up to one month if needed.
- b. Addition of TCA and Neutralization Solution dilutes protein sample concentration down to 80% compared to the original concentration. Correct values by dividing results using 0.8 as dilution factor.
- c. For further sample analysis, if reaction buffer capacity is 100 mM or stronger, sample volume up to 50  $\mu$ l may be used if total reaction volume is 100  $\mu$ l. For systems with weak buffer capacities, lower sample volume must be used to maintain optimum pH in the reactions.



**Figure: (a) Effect of TCA amount on deproteinization:**

Aliquots of pooled human serum (off the clot, 100  $\mu$ l) were treated with different amounts of TCA following the kit protocol. Protein concentrations were measured using BCA Protein Assay Kit II (Cat. # K813). Estimated protein concentration before deproteinization using BCA method: 7.9 g/dl. More than 99% of protein was removed using less than 10  $\mu$ l of TCA/100  $\mu$ l human serum. **(b) Comparison of analysis of lactate:** Untreated human serum samples (100  $\mu$ l) or samples deproteinized using the kit protocol were analyzed for lactate concentration using Lactate Colorimetric Assay Kit II (K627).

## VII. Related Products:

Deproteinizing Sample Preparation Kit (K808)

BCA Protein Quantitation Kit (K812)

BCA Protein Assay Kit - Reducing Agent Compatible (K818)

Albumin (BCG) Assay Kit (Colorimetric) (K554)

10kDa Spin Column (1997)

BCA Protein Assay Kit II (K813)

Glucose Colorimetric/Fluorometric Assay Kit (K606)

Lactate Colorimetric Assay Kit II (K627)

**FOR RESEARCH USE ONLY! Not to be used on humans.**