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Product Information

Deproteinizing Sample Preparation Kit II

Catalog Number **MAK342**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

The study of biological fluids, 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) degrade or interfere with low molecular-weight metabolites. Therefore, sample deproteinization is often required. Chemical deproteinization (e.g., trichloroacetic acid, TCA) is one of the most utilized methods to accomplish sample deproteinization.

The 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 the 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.

Components

The kit is sufficient for 200 assays.

TCA Catalog Number MAK342A	3 mL
Neutralization Buffer Catalog Number MAK342B	4 mL

Reagents and Equipment Required but Not Provided.

- Microcentrifuge capable of RCF ≥12,000 × *g*
- Microcentrifuge tubes
- Pipetting devices and accessories

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store the kit at room temperature. Place kit components on ice to chill before use. There may be some precipitation in the Neutralization Buffer. Shake bottle gently a few times to resuspend before using.

Procedure

Note: The following procedure can be proportionally scaled up or down for preparation of larger or smaller sample volumes.

Protein Precipitation – Serum and other high protein concentration samples

1. Pipette 100 µL of sample and 15 µL of ice cold TCA in a 1.5 mL microcentrifuge tube.
2. Vortex briefly to mix well.
3. Place on ice for 15 minutes.
4. Centrifuge at 12,000 × *g* for 5 minutes.
5. Accurately transfer 100 µL of the supernatant to a fresh tube.

Note: 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.

Protein Precipitation – Samples with protein concentration less than ~25 mg/ml (e.g. tissue lysate, cell lysate, and yeast lysate)

1. Pipette 150 µL of sample and 15 µL of ice cold TCA in a 1.5 mL microcentrifuge tube.
2. Vortex briefly to mix well.
3. Place on ice for 15 minutes.
4. Centrifuge at 12,000 × *g* for 5 minutes.
5. Accurately transfer 150 µL of the supernatant to a fresh tube.

Note: 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.

Sample Neutralization

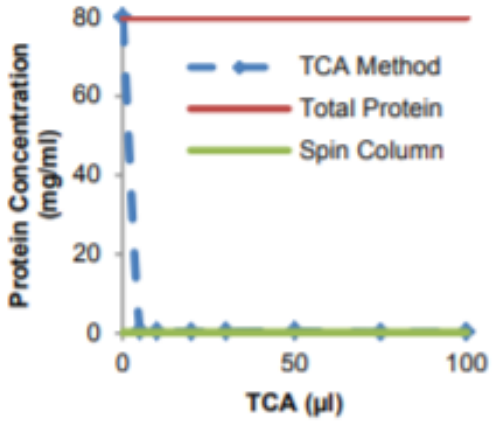
1. To neutralize excess TCA, add 10 µL of cold Neutralization Buffer to the collected supernatant.
2. Mix well. Vent sample tube as there may be formation of CO_2.
3. Place sample on ice for 5 minutes. Samples are now deproteinized and neutralized, and can be directly used in a variety of assays.

Notes:

1. Addition of TCA and Neutralization Buffer dilutes protein sample concentration down to 80% compared to the original concentration. Correct values by dividing results using 0.8 as dilution factor.
2. For further sample analysis, if reaction buffer capacity is 100 mM or stronger, sample volume up to 50 µL may be used if total reaction volume is 100 µL. For systems with weak buffer capacities, lower sample volume must be used to maintain optimum pH in the reactions.

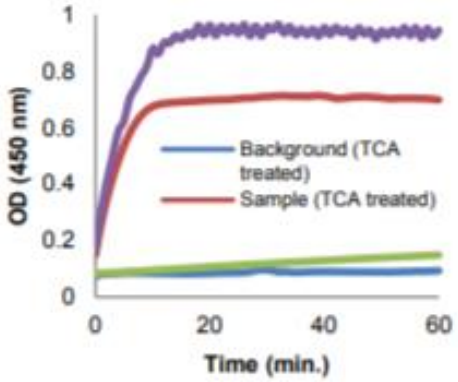
Results

Figure 1.
Effect of TCA amount on deproteinization



Aliquots of pooled human serum (off the clot, 100 µL) were treated with varying amounts of TCA following the kit procedure. Protein concentrations were measured using a BCA protein assay method. Estimated protein concentration before deproteinization using BCA method: 7.9 g/dL. More than 99% of protein was removed using less than 10 µL of TCA/100 µL human serum.

Figure 2.
Lactate Assay



Untreated human serum samples (100 µL) and samples deproteinized using the kit protocol were analyzed for lactate concentration using the Lactate Assay Kit II (MAK065).

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