PI-0103

Sample Deproteinizing Kit (PCA)

(Sufficient for 200 samples, Store at Room Temperature)

Background Information:

It is a fundamental requirement in biochemistry and related fields to be able to remove those constituents of the sample under investigation which will interfere with analysis in any of several ways, both physical and chemical. When the analyte in question is a small molecule, the presence of macromolecules such as proteins, nucleic acids and carbohydrates can seriously impair analysis. Proteins are usually removed by precipitation using one of several chemicals, acetone, perchloric acid (PCA), metaphosphoric acid (MPA), sulfosalicylic acid (SSA) or trichloroacetic acid (TCA). Like kit PI-0102, this kit is designed to remove protein from samples which may interfere with various analyses. PCA deproteinization methods have been successfully used in the preparation of samples prior to quantitation of an array of small molecules, including glycogen, ATP, cAMP, glutathione and various antioxidants. AkrivisBio's Deproteinizing Sample Preparation Kit utilizes a PCA precipitation method, providing a unique tool to prepare samples for the analysis of a variety of small molecules. Using the kit, proteins are precipitated, excess PCA is removed then samples are neutralized. Samples prepared using this kit can be directly used in a wide variety of bioassays. The method is easy, convenient, and can be used for the preparation of a large number of samples.

Kit Contents:

PCA	20 ml	WM	PI-0103-A
Neutralization Solution	4 ml	NM	PI-0103-B

Storage and Handling:

This kit may be stored at room temperature. Protein precipitation works best cold, so place kit components on ice to chill prior to use. The neutralization solution should be shaken briefly before use to resuspend the fine precipitate present in the bottle.

Deproteinizing Sample Preparation Protocol:

This procedure may be scaled to handle a wide range of sample sizes.

1. Protein Precipitation:

For samples with a protein concentration < ~20 mg/ml (tissue homogenate, cell lysate, urine, etc.), to 500 µl of sample, add 100 µl of ice cold PCA in a 1.5 ml microfuge tube, vortex and place on ice for 5 min. Centrifuge at 13,000 x g for 2 min. Transfer precisely 480 µl of the supernatant to a fresh tube. For serum and other samples with a high protein concentration, to 400 µl of sample, add 100 µl of ice-cold PCA, place on ice for 5 min. Centrifuge at 13,000 x g for 2 min. Transfer precisely 380 µl of the supernatant to a fresh tube. Depending on the nature of the analyte, PCA treated samples may be stored at -70°C for up to a month.

2. Sample Neutralization:

To the clear supernatant obtained after centrifugation, add 20 µl of shaken ice-cold Neutralization Solution and vortex briefly to neutralize the sample and precipitate excess PCA. There will be some gas (CO_2) evolution so vent the sample tube. Place on ice for 5 min then spin briefly. Samples are now deproteinized, neutralized, and PCA has been removed. The samples may now be used in a variety of assays directly.

Note 1: Deproteinized samples have been diluted to 80% of the original concentration (quantitation results should be divided by 0.8 to correct measured values back to original sample concentrations). For serum samples, the dilution is to 76% so divide assay values by 0.76 to correct values to original sample concentrations.

Note 2: For further analysis of samples, if assay buffer is 0.1 M or stronger, samples up to 50 µl may be used directly in 100 µl assay reactions. If lower concentration buffers are used in the assay, correspondingly smaller sample volumes should be used to maintain assay reaction pH without significant changes.

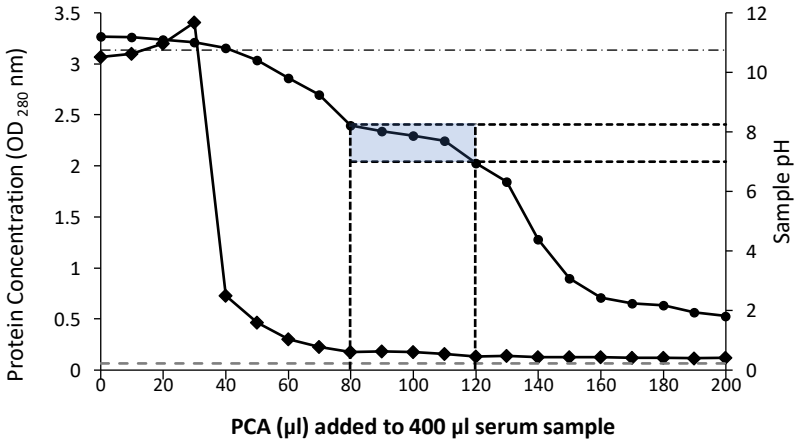

Figure: Deproteinization of serum samples. Different amounts of PCA were used to deproteinize 400 µl of serum following the kit protocol. Protein concentration remaining in solution OD_{280} (♦) and sample pH (●) was measured. Samples (neat serum; serum filtered through a 10kD MW cutoff filter and PCA treated) were diluted 20 times for convenience of measuring OD at 280 nm. A separate aliquot of the same PCA treated samples was mixed 1:1 with a pH 7.75 assay buffer and the pH of the mixture determined. The figure shows that sample/PCA ratio errors up to 20% can be tolerated with the resulting assay pH holding within approximately 0.5 pH units of the target assay pH. Using a 10 kDa MW cutoff filter removes approximately 98 % of protein and PCA precipitation at a PCA/sample ratio of 1:4 removes approximately 95 % of protein present.

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