

Deproteinizing Sample Preparation Kit (#BN01024)

(Catalog #BN01024; Store at +4° C)

I. Introduction:

The presence of protein and various enzyme activities frequently interferes with the analysis of small molecules in biological samples. Many bioassays require removal of proteins from samples prior to analysis. Among the deproteinization protocols developed over the last half century, perchloric acid (PCA) precipitation has been extensively used in many different sample preparation procedures, since not only does it remove most of the protein present but it also functions to stabilize many of the small molecule analytes. PCA deproteinization methods have been successfully used in the preparation samples prior to quantitation of an array of small molecules, including glycogen, ATP, cAMP, glutathione, antioxidants, etc. The newly designed **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, 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.

II. Kit Contents:

Components	BN01024	Cap Code	Part Number
PCA	20 ml	WM	BN01024-1
Neutralization Solution	4 ml	NM	BN01024-2

III. Storage and Handling:

Store kit at +4° C. Keep kit components ice cold while in use. There may be some precipitation in the neutralization buffer. Shake bottle gently a few times to resuspend before using.

IV. Deproteinizing Sample Preparation Protocol:

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

1. Protein Precipitation:

For biological samples with protein concentration less than ~ 20 mg/ml (tissue homogenate, cell lysate, urine, etc.), take 500 µl of sample, mix with 100 µl of ice cold PCA in 1.5 ml microfuge tubes, vortex briefly to mix well, place on ice for 5 min. Centrifuge at 13,000 x g for 2 min. Accurately transfer 480 µl of the supernatant to a fresh tube.

For serum and other very high protein concentration samples, take 400 µl of sample and mix with 100 µl of ice-cold PCA, place on ice for 5 min. Centrifuge at 13,000 x g for 2 min. Accurately transfer 380 µl of the supernatant to a fresh tube.

Depending on the nature of the analyte, the samples in PCA may be frozen at -70° C for up to a month for storage at this stage.

2. Sample Neutralization:

Add 20 µl of ice-cold Neutralization Solution (resuspend the fine precipitate) and mix to neutralize the sample and precipitate excess PCA. There may be some gas (CO₂) evolution so vent the sample tube. Place on ice for 5 min. Spin briefly (~ 1 - 2 min). Samples are now deproteinized, neutralized, and PCA has been removed. The samples may now be used in a variety of assays directly.

Note 1: The 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.

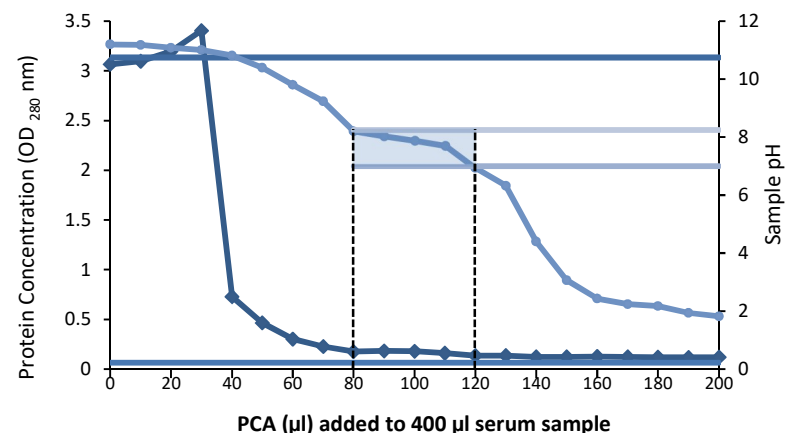


Figure: Deproteinization of serum samples: Different amounts of PCA were used to deproteinize 400 µl of serum following the kit protocol. Protein concentration remaining in solution OD₂₈₀ and sample pH was measured. Samples (neat serum; serum filtered through a 10kD MW cutoff; and PCA treated) were diluted 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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