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# Product Information

**Deproteinizing Sample Preparation Kit**

Catalog Number **MAK341**
Store at Room Temperature

## TECHNICAL BULLETIN

### Product Description

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 sample preparation prior to quantitation of an array of small molecules, including glycogen, ATP, cAMP, glutathione, antioxidants, etc.

The 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, and the samples are then 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.

### Components

The kit is sufficient for 200 assays.

| | |
|---|---|
| PCA<br>Catalog Number MAK341A | 20 mL |
| Neutralization Buffer<br>Catalog Number MAK341B | 4 mL |

### Reagents and Equipment Required but Not Provided.

- Microcentrifuge capable of RCF ≥13,000 × *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 and storage at room temperature is recommended. 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 – Biological samples with protein concentration less than ~20 mg/mL (tissue homogenate, cell lysate, urine, etc.)

1. Pipette 500 µL of sample and 100 µL of ice cold PCA in a 1.5 mL microcentrifuge tube.
2. Vortex briefly to mix well.
3. Place on ice for 5 minutes.
4. Centrifuge at 13,000 × *g* for 2 minutes.
5. Accurately transfer 480 µL of the supernatant to a fresh tube.
6. Depending on the nature of the analyte, the samples in PCA may be stored at –70 °C for up to a month.

Protein Precipitation – Serum and other high protein concentration samples

1. Pipette 400 µL of sample and 100 µL of ice-cold PCA into a 1.5 mL microcentrifuge tube.
2. Vortex briefly to mix well.
3. Place on ice for 5 minutes.
4. Centrifuge at 13,000 × *g* for 2 minutes.
5. Accurately transfer 380 µL of the supernatant to a fresh tube.
6. Depending on the nature of the analyte, the samples in PCA may be stored at –70 °C for up to a month.

Sample Neutralization

1. Add 20 µL of ice-cold Neutralization Solution (resuspend the fine precipitate) to supernatant.
2. Mix to neutralize the sample and precipitate excess PCA. There may be some gas ($CO_2$) evolution so vent the sample tube.
3. Place on ice for 5 minutes.
4. Centrifuge briefly (1–2 minutes).
5. Samples are now deproteinized, neutralized, and PCA has been removed. The samples may now be used in a variety of assays directly.

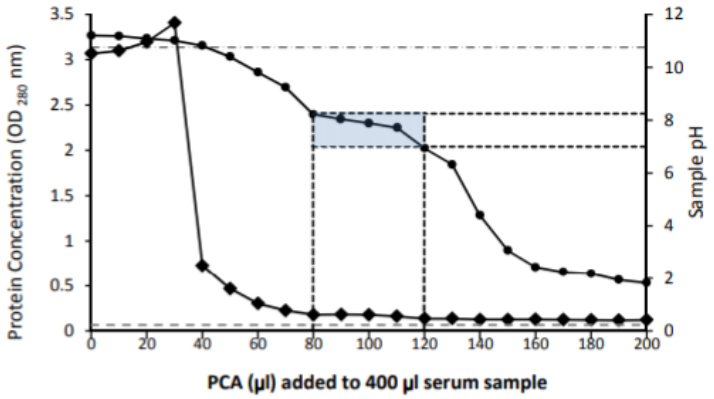
Notes:

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.
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.

**Results**

**Figure 1.**

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 $A_{280}$ (♦) and sample pH (•) was measured. Samples (neat serum, serum filtered through a 10 kDa MW cutoff, and PCA treated) were diluted 20 times for convenience of measuring $A_{280}$. 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 ~0.5 pH units of the target assay pH. Using a 10 kDa MW cutoff filter removes ~98% of protein and PCA precipitation at a PCA/sample ratio of 1:4 removes ~95 % of protein present.

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