

## ab284939 – Deproteinizing Sample Preparation Kit - PCA

For preparing samples for the analysis of a variety of small molecules, proteins are precipitated, excess PCA (perchloric acid) is removed, samples are neutralized.

For research use only - not intended for diagnostic use.

- b) For further analysis of samples, if assay buffer is 0.1 M or stronger, samples up to 50  $\mu\text{L}$  may be used directly in 100  $\mu\text{L}$  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.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284939>

### Storage and Stability

On receipt entire assay kit should be stored at room temperature, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

### Materials Supplied

Item	Quantity	Storage Condition
Perchloric Acid Solution	20 mL	Room temperature
Neutralization Buffer I	4 mL	Room temperature

PLEASE NOTE: Neutralization Buffer I was previously labelled as LOX Probe, and Perchloric Acid Solution as PCA. The composition has not changed."

### Kit Protocol

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

#### 1. Protein Precipitation:

1. **For biological samples with protein concentration less than ~20 mg/mL** (tissue homogenate, cell lysate, urine, etc.), take 500  $\mu\text{L}$  of sample, mix with 100  $\mu\text{L}$  of ice cold Perchloric Acid Solution 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.
2. Accurately transfer 480  $\mu\text{L}$  of the supernatant to a fresh tube.
3. **For serum and other very high protein concentration samples**, take 400  $\mu\text{L}$  of sample and mix with 100  $\mu\text{L}$  of ice-cold Perchloric Acid Solution, place on ice for 5 min.
4. Centrifuge at 13,000 x g for 2 min.
5. Accurately transfer 380  $\mu\text{L}$  of the supernatant to a fresh tube.

Depending on the nature of the analyte, the samples in Perchloric Acid Solution may be frozen at  $-70^{\circ}\text{C}$  for up to a month for storage at this stage.

#### 2. Sample Neutralization:

1. Add 20  $\mu\text{L}$  of ice-cold Neutralization Buffer I (resuspend the fine precipitate) and mix to neutralize the sample and precipitate excess Perchloric Acid Solution. There may be some gas ( $\text{CO}_2$ ) evolution so vent the sample tube.
2. Place on ice for 5 min.
3. Spin briefly (~1- 2 min). Samples are now deproteinized, neutralized, and Perchloric Acid Solution has been removed. The samples may now be used in a variety of assays directly.

#### $\Delta$ Notes:

- a) 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.

### Technical Support

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