Deproteinization protocol

The analysis of small molecules in biological samples is frequently hindered by the presence of protein and various enzyme activities. Many bioassays require removal of protein from samples prior to analysis. Perchloric acid (PCA) precipitation is one of the most extensively used deproteinization protocols since it not only removes most of the protein present in the sample but it also functions to stabilize many of the small molecule analytes.

PCA deproteinization has been successfully used in the preparation of samples prior to quantitation of an array of small molecules, including glycogen, ATP, cAMP, glutathione, antioxidants, etc.

Materials

- 4 M perchloric acid (PCA), ice cold
- 2 M KOH, ice cold (neutralizing agent)
- Microcentrifuge (set 4°C and previously cooled down)
- Ice
- Microcentrifuge tubes

Protein precipitation

1. Prepare sample as specified in the product protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.
2. Add PCA to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well.

   High protein concentration samples might need more PCA.

3. Incubate samples on ice for 5 min. Centrifuge samples at 13,000 rpm for 2 min in a cold centrifuge. Transfer the supernatant to a fresh tube.

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

Sample neutralization

1. Neutralize supernatant by adding ice-cold 2 M KOH that equals 34% of the supernatant to your sample (for example, add 34 µL of 2 M KOH to 100 µL of sample) and briefly vortex. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO_2) evolution so vent the sample tube.
2. After neutralization it is very important that the pH equals 6.5-8.0 (use pH paper to test 1 µL of sample. If necessary, adjust the pH with 0.1 M KOH or PCA.
3. Spin samples at 13,000 rpm for 15 min in a cold centrifuge. Collect supertanant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples may now be used directly for the relevant assays.

Sample recovery

The deproteinized samples will be diluted from the original concentration. To calculate the dilution factor of your final sample, simply apply the following formula:

$$\% \text{ original concentration} = \frac{\text{initial sample volume}}{(\text{initial sample volume} + \text{volume PCA} + \text{volume KOH})} \times 100$$