PI-0102

Sample Deproteinizing Kit (TCA)

(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). There are advantages to the use of one or the other depending upon what type of further analysis will be performed. TCA is useful since it can precipitate both proteins and nucleic acids and is easily neutralized, and many small molecules are quite stable in its presence. Once protein precipitation has been accomplished, the precipitate is spun down to form a tight pellet and the supernatant is removed for further processing. A simple neutralization using an alkaline material such as NaOH or KOH is used to return the pH to generally a neutral range where the analyte in question is most stable. AkrivisBio's TCA Precipitation Kit is a simple quick and easy way to prepare a variety of biological samples.

Kit Contents:

100% TCA	3 ml	NM	PI-0102A
Neutralization Solution (~8 M)	4 ml	NM	PI-0102B

Storage Conditions and Reagent Preparation:

Kit may be stored at room temperature. Protein precipitation works best cold, so place TCA on ice prior to using. Keep Neutralization Solution tightly closed. It will absorb CO_2 resulting in the formation of insoluble carbonates reducing the neutralizing power of the solution.

Protein Precipitation Protocol:

1. Protein Precipitation.

Add ice cold 100% TCA to samples on ice to a final concentration of 8-12%. A convenient ratio is 10 µl per 100 µl of sample. This is conveniently done in a 0.5 – 2 ml microcentrifuge tube. Allow the precipitation to proceed on ice for 15-30 minutes. Centrifuge at 16,000 x g for 5 min. Transfer supernatant to a fresh tube without disturbing the protein pellet.

2. Neutralization:

10% TCA is slightly more than 600 mM. To neutralize TCA, add 5 µl of cold Neutralization Solution to 100 µl of supernatant. Mix and vent to release pressure from CO_2 forming in the tube. Add 5 µl more Neutralization Solution, mix and vent again. pH can be checked using a few µl on a pH test strip. Generally, a pH of 6-9 is satisfactory for the vast majority of samples. Allow samples to remain on ice for 2-5 minutes to allow any further gas formation to subside. Samples are now ready to be used.

Note: AkrivisBio Assays typically include 100 mM buffers. This buffering capacity is sufficient for up to 50 µl of samples prepared with this kit. Be cautious with kits from other manufacturers who frequently use buffers of a lower concentration. You may be required to use smaller sample sizes for their kits to function correctly, resulting in significantly lower sensitivity toward the analyte of interest.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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