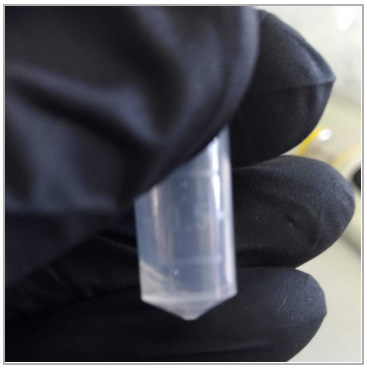

MAY 17, 2023

DOI:
dx.doi.org/10.17504/protocols.io.5jyl8j74dg2w/v1

Protocol Citation: Angelo José Rinaldi 2023. Simple TCA/acetone protein extraction protocol for proteomics studies.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8j74dg2w/v1

Protocol status: Working
We use this protocol and it's working

Created: Apr 29, 2023

Last Modified: May 17, 2023

PROTOCOL integer ID: 81176

Simple TCA/acetone protein extraction protocol for proteomics studies.

Angelo José Rinaldi[1]

[1]Universidade Federal de Viçosa

Angelo José Rinaldi

ABSTRACT

Protein extraction with TCA (trichloroacetic acid) and acetone is a widely used method to precipitate proteins from biological samples. This method is useful for obtaining proteins of interest for proteomic studies or biochemical analyses. Here are some reasons why TCA/acetone protein extraction is important:
Selective Protein Precipitation: TCA/Acetone is effective in selective protein precipitation, allowing the removal of contaminants and unwanted components present in the sample. This is especially important in proteomic studies, where protein purity is critical for subsequent analyses.
Removal of lipids and carbohydrates: TCA/acetone is efficient in removing lipids and carbohydrates, which can interfere with proteomic and biochemical analyses. These compounds are precipitated together with the proteins, facilitating their removal and improving the quality of the samples.
Preservation of protein structure and function: The use of TCA/acetone for protein extraction can preserve protein structure and function. These precipitating agents do not denature proteins, allowing analysis of proteins in their native or active form.
Simplified sample processing: Protein extraction with TCA/acetone is relatively simple and fast, providing a convenient approach to processing biological samples. This is especially advantageous when working with large numbers of samples or in situations where time is critical.
In summary, protein extraction with TCA/acetone is an important and versatile method that allows obtaining high quality proteins for various analyzes and studies. Its efficiency in removing contaminants, preserving protein structure and simplicity make it an essential technique in biomedical and biotechnological research.

MATERIALS

Necessary materials:

1) Biological sample containing the proteins of interest
2) TCA (trichloroacetic acid) concentrate
3) Acetone 80% (cooled to -20°C)
4) Detergent (SDS 10%)
5) centrifuge
6) Microcentrifuge tubes
7) Ice
8) Tris-HCL 6.8 (tris(hydroxymethyl)aminomethane)

1 Prepare a 20% TCA solution by adding 200g of TCA in 800ml of distilled water. Be sure to do this in a well-ventilated area, wearing gloves and eye protection, as TCA is corrosive.

2 Add the biological sample (approximately 300 mg) to a 2 mL microcentrifuge tube.

3 Add a small amount of detergent (10% SDS) to the sample, if necessary, to break down cell membranes and facilitate protein release. The exact amount of detergent depends on the sample type and must be optimized experimentally.

4 Transfer the homogenized sample to a new microcentrifuge tube and centrifuge at high speed (eg 13,000 rpm) at 4°C for 20 minutes to remove cell debris and nuclei.

5 Discard the supernatant carefully, without disturbing the pellet formed at the bottom of the tube.

6 Add concentrated TCA (20% final) to the pellet containing the proteins, in a ratio of 4 parts of TCA to 1 part of the sample. For example, if you have 200 µl of sample, add 800 µl of TCA.

7 Incubate the sample on ice for 20-30 minutes to allow protein precipitation.

8 Centrifuge the sample at 4°C for 20 minutes at high speed (eg 13,000 rpm).

9 Carefully discard the supernatant and add 1 ml of 80% acetone (cooled to -20°C) to the pellet. Make sure the acetone completely covers the pellet.

Note: Wash the pellet with 80% acetone, 2x.
To do this, simply add 80% acetone and quickly stir using a vortex tube stirrer and centrifuge at 13,000 RPM for 5 min.

10 Incubate the sample on ice for 5 minutes to allow for further protein precipitation.

11 Centrifuge the sample at 4°C for 20 minutes at high speed (eg 13,000 rpm).

12 Carefully discard the supernatant and dry the protein pellet at room temperature.

13 The dried protein pellet can be resuspended in an extraction buffer (10% SDS with 6.8 tris-HCl) suitable for further analysis or apply 10 µL onto the SDS-PAGE electrophoresis gel.