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Salivary Protein Extraction and Precipitation Protocol

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We use this protocol and it's working

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Abstract

This protocol outlines a method for extracting and precipitating proteins from human saliva samples. The procedure involves sample preparation, protein precipitation using trichloroacetic acid (TCA), acetone washing, and protein quantification via the Bradford assay.

Materials

Saliva Samples: Minimum of 5 mL per sample, collected in 15 mL Falcon tubes. Milli-Q Water. Trichloroacetic Acid (TCA), 20% (w/v). Cold Acetone, 90% (v/v). Cold Acetone, 80% (v/v). Sodium Hydroxide (NaOH), 0.2 M. 2D Buffer: Urea 7 M, Thiourea 2 M, CHAPS 4%. Bradford Reagent. Equipment: Centrifuge capable of reaching 8000 rpm with temperature control. Vortex Mixer. Pipettes and Tips. Microcentrifuge Tubes. -20°C and -40°C Freezers.

Saliva Sample Preparation

1 Collect a minimum of 5 mL of saliva into a 15 mL Falcon tube.

2 Add an equal volume of Milli-Q water to the saliva sample (1:1 v/v ratio).

3 Vortex the mixture for 30 seconds to ensure homogeneity.

4 Centrifuge at 3500 rpm for 25 minutes at 4°C.

5 Carefully collect the supernatant and aliquot into four 1000 µL portions.

6 Store the aliquots at -40°C until further analysis.

Protein Precipitation

7 To each 1000 µL saliva aliquot, add 1000 µL of 20% TCA solution.

8 Vortex the mixture for 30 seconds.

9 Incubate the samples at -20°C for 30 minutes to facilitate protein precipitation.

10 Centrifuge at 5000 rpm for 30 minutes at 4°C.

11 Discard the supernatant carefully without disturbing the pellet.

12 Add 200 µL of cold 90% acetone to the pellet.

13 Vortex for 30 seconds to wash the pellet.

14 Incubate at -20°C for 20 minutes.

15 Centrifuge at 8000 rpm for 5 minutes at 4°C.

16 Discard the supernatant.

17 Add 200 µL of cold 80% acetone to the pellet.

18 Vortex for 30 seconds.

19 Incubate at -20°C for 10 minutes.

20 Centrifuge at 8000 rpm for 10 minutes at 4°C.

21 Carefully discard the supernatant completely.

22 Allow the pellet to air-dry for 5 minutes to remove residual acetone.

Protein Quantification

23 Resuspend the dried protein pellet in 10 µL of 0.2 M NaOH.

24 Incubate the solution for 2 minutes at room temperature to solubilize the proteins.

25 Add 50 µL of 2D buffer (Urea 7 M, Thiourea 2 M, CHAPS 4%) to the solution.

26 Vortex the mixture for 30 seconds to ensure complete dissolution.

27 Collect the supernatant for protein quantification.

28 Determine the protein concentration using the Bradford assay according to the manufacturer's instructions.

Protocol references

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