Minute™ Native Protein Precipitation Kit from Saliva

Cat. No. SV-043

Description

Human saliva is a readily available source for identifying biomarkers for diagnosis of local and systemic diseases. Proteins in saliva contain biomarkers that can be identified by proteomics for the diagnosis of many diseases such as Sjögren's syndrome, squamous cell carcinoma, diabetes, breast cancer, dental caries, periodontitis, systemic sclerosis and bleeding oral cavity etc. Historically, the most common method for extracting proteins from saliva is trichloroacetic acid (TCA) precipitation. Although relatively simple, rapid and effective, TCA precipitation suffers a major drawback e.g. the precipitated proteins are denatured and insoluble in detergent-free aqueous solution. That limits the downstream application of extracted proteins. By contrast, the native protein extraction kit is designed to overcome the disadvantage of TCA method using a propitiatory protein precipitation reagent that extracts native proteins from saliva. The method is also very simple and rapid with high yield. The precipitated proteins can be readily dissolved in detergent-free aqueous solutions.

Applications

The extracted proteins can be used for SDS-PAGE, ELISA, immunoprecipitation, 2D gel analysis, mass spectrometry, enzymatic assays and other applications.

Kit Components (20 prep)

1.	SV Reagent	20 ml
2.	Filter Cartridge	20
3.	Collection tube	20

Additional Materials Required

Table-top micro centrifuge with maximum speed of 14,000-16000 x g.

Shipping and Storage: Ship at ambient temperature and store at RT.

Protocol

Note: Perform all centrifugation steps at 4°C. It is recommended to add proteinase inhibitors to saliva sample prior to use. The starting sample volume can be scaled down to as low as 50 µl.

1. Transfer 0.6 ml fresh or frozen saliva to a filter cartridge in a collection tube. Cap the filter and centrifuge at 16,000 X g for 10 min. This step instantly converts viscous saliva into non-viscous liquid and removes cell debris and insoluble materials in the sample.
2. Transfer 0.5 ml supernatant to a fresh 1.5 ml tube and add 1.0 ml protein precipitation reagent to the tube (saliva to reagent ratio is 1:2). Mix well by vortexing vigorously for 10 seconds.
3. Incubate the tube at 4°C for 30 min. Centrifuge at 16,000 X g for 10 min to bring down precipitated protein. Remove the supernatant completely. Centrifuge briefly at 16,000 X g to bring down reagent on the wall of the tube. Remove supernatant completely.

4. Resuspend the pellet in100-200 µl buffer of your choice that can be detergent-free or detergent containing depending upon the downstream applications. For example: if the downstream application is 2D gel or mass spectrometry analysis, the salt in the protein prep will need to be removed by a desalting column or dialysis. The final protein yield is typically 1-2 mg/ml. Resuspended protein can also serve as a starting material for isolation of exosomes by other methods.

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