Minute™ Protein Extraction Kit for Hair and Nails

Catalog number: HD-021

Description

Proteins account for about 80% of keratinized animal tissues that include but not limited to hair, nail, horn and wool. These tissues contain mainly hard α-keratins made of several distinctive types of protein with molecular weight ranging from 10-135 Kda. Majority of the proteins are ranging from 10-65 Kda. Several methods have been reported for protein extraction from keratinized animal tissues. The extraction buffer usually contains high concentration of urea, thiourea, guanidine hydrochloride or a combination of these strong denaturants. Protein extraction with high concentration denaturants is relatively effective but the concentration of extracted protein is usually low due to larger extraction buffer/tissue ratio. Extracted proteins need to be concentrated prior to further analysis. The presence of high concentration of denaturants may also interfere with downstream application. HD-021 is designed to effectively extract proteins from keratinized animal tissues without using strong denaturants mentioned above. The extracted protein can be directly loaded onto SDS-PAGE without concentration. The extraction buffer of this kit contains 0.5% SDS and other chemicals. Extracted protein concentration is 1-2 mg/ml.

Application

This kit is designed to extract proteins from keratinized animal tissues for applications such as SDS-PAGE, immunoblottings, ELISA and other applications.

Kit Components

1. 15 ml buffer A
2. 1.5 ml buffer B
3. 20 filter cartridges
4 20 collection tubes with cap

Shipping: This kit is shipped at ambient temperature
Storage: Store the kit at room temperature

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Important Product Information

The use of protease inhibitors is not necessary prior to extraction. However if downstream application takes significant amounts of time or the protein extract will be stored for longer period of time, addition of protease inhibitor to buffer A is recommended. For determination of protein concentration reducing agent compatible method is recommended. For protein phosphorylation studies, cocktails of phosphatase inhibitors need to be added to buffer A prior to use.

Additional Materials Required

2-Mecaptolethanol (2-ME)
Table-Top Microcentrifuge

Protein Extraction Procedures

Following procedures are for 4-5 mg of animal hair or 8-10 mg nails and horns/sample.
If smaller amount of tissue is used reduce the reagents proportionately

1. Prior to protein extraction, cut keratinized tissue into smaller pieces (1 to 2 mm)
2. Place the tissue in a 1.5-2.0 ml microcentrifuge tube. Add 400 µl buffer A into the tube followed by addition of 20 µl 2-ME to the tube.α
3. Disturb the tissue with a pipette tip and make sure the tissue is wet and submerged in buffer A. Cap the tube and incubate at 55^{o}C in a water bath for 24h.
4. After incubation add 40 µl buffer B to the tube, vortex briefly and pour all content in the tube into a filter cartridge in a collection tube. The tissue can be pushed to the filter cartridge with the aid of a 200 µl pipette tip.
5. Centrifuge at top speed in a table top microcentrifuge for 2-3 min and transfer the supernatant of the flow through to a fresh tube. This is extracted proteins. Determine protein concentration by Bradford method or using a reducing agent compatible commercial kit.