Protein Extraction Kit from Gel Slices

Cat. #: P519-20 (20 reactions); P519-80 (80 reactions)

Storage: at room temperature

Shelf Life: 12 months

Product Description

Extract protein from gel in < 10 minute (hands on) with high yield. The recovery efficient is good in the range of 10~200 kDa. Multiple gel pieces can be processed in one tube to yield very high concentration protein. The final elution volume is 10 to 200 uL. This kit does not need any special devices.

Extracted proteins can be used for MALD-MS analysis, immunization of animals, protein-protein interaction and protein-nucleic acid interaction studies etc.

This product is for research use only.

Product Contents (Store at room temperature)

Component	Amount	
	Cat. #: P920-20	Cat. #: P920-80
Protein extraction filter cartridges	20	80
Collection tubes with cap (2 mL)	20	80
Microcentrifuge tube (200 uL)	20	80
Extraction powder	2g	8g
Micro pestle	4	16

Protocol:

1. After separation of protein samples by SDS-PAGE or 2-D gel, the gel can be stained by a positive staining method such as standard **Coomassie blue** staining, or a negative staining method (see references below). If Coomassie blue staining is used, be sure to stain the gel briefly (**use shortest staining time** to reveal the protein band of interest, usually **less than 5 minutes**).

   Rinse the gel with **ddH_2O at least 3 times** if the gel is stained with Coomassie.

2. Cut the band of interest out of the gel with a blade and trim away excessive gel. Remove excessive liquid associated with the gel by touching with filter paper. Place 1-2 excised gel pieces at the bottom of 200 µL tube provided (the tube can accommodate multiple pieces of excised gels).

3. Using the flat end of the pestle, add extraction powder (about 1/4 to 1/3 the volume of the gel) to the gel at bottom of the tube. Add elution buffer of your choice (see below) to the tube (**20 µL/piece** of gel).

4. Insert the sharp fork of pestle to the bottom of the tube. Twist or punch back and forth repeatedly to mince the gel to fine slurry. It takes about 1-2 min. While the pestle still in the tube add **20-50 µL/gel** piece elution buffer to the tube and make sure the gel slurry is covered by elution buffer.

   Note: the pestle is reusable. Rinse with distilled water and dry it with paper towel.

5. Cap the tube and incubate at **94ºC** in a PCR machine for **5-10 minutes**. Vortex the tube briefly and continue to incubate for another **5-10 minutes**. Longer incubation will increase protein yield. The capped tube can also be left at 4°C overnight.

6. Place a filter cartridge in a collection tube.

   Open the cap of the 200 µL tube , trim the cap off with a blade or a pair of scissors, and insert the opening end of 200 µL tube to the filter cartridge. Centrifuge in a microfuge at top speed for 2 minutes. Discard the filter and save eluted protein in collection tube.

Choice of elution buffers

1. The choice of elution buffer depends on how gel is stained and the downstream applications.
2. If the stain contains fixing agent such as methanol and acetic acid, an elution buffer containing 0.1-0.5% SDS or acid labile surfactants is recommended.
3. If the gel is un-stained or negatively stained the protein can be eluted with ddH2O or an elution buffer containing formic acid/water/2-propanol (1:3:2 v/v/v).

References of Negative Staining:

1. Cohen SL and Chait BT. Anal Biochem. 1997 May 1;247(2):257-67.
2. Ortiz ML and Calero M etc. FEBS Lett. 1992 Jan 27;296(3):300-304.

Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.