Minute™ Protein/Nucleic Acid Extraction Kit from Gels

Catalog number: PN-019

Description

Passive elution and electro elution are the most common methods for extracting proteins and/or nucleic acid from polyacrylamide/agarose gels. Protein extraction by passive elution usually takes overnight incubation. Eluted proteins are significantly diluted and require further concentration. Protein extraction by electro elution is faster (30 min to 2 h) than passive elution but it requires a special elution device that works less effectively for larger proteins (>70 Kda). The electro buffer usually contains detergent and other chemicals at a concentration that may interfere with downstream applications. Here we feature a rapid and instrument-free protein/nucleic acid extraction kit. Proteins/nucleic acid can be extracted from gels in <10 min with high yield. The elution buffer can be a detergent-containing buffer or pure water depending upon methods of gel staining. The elution volume is between 10 to 200 µl. Multiple gel pieces can be processed in a single tube and the final protein concentration is relatively high.

Application

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

Kit components(20T):

1. 20 filter cartridges with 2.0 ml collection tubes
2. 20 microfuge tubes (200 µl)
3. 2 gram extraction powder
4. Micro pestle X 2

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

Additional Materials Required: A Table-Top Microcentrifuge

Protocols

A. Protein extraction from polyacrylamide gel

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

1864 Berkshire Ln N, Plymouth, MN 55441 | p: (952)400-6898 | f: (952)400-6889
info@inventbiotech.com | www.inventbiotech.com

method (see references below). The gel is de-stained to reveal protein band(s) of interest. Rinse the gel with ddH_2O at least 3 times if the gel is stained with Coomassie blue staining.

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. Add extraction powder (about 1/4 to 1/3 the volume of the gel) to the gel at bottom of the tube using the flat end of the pestle. Add elution buffer of your choice (see below) to the same tube (20 µl/piece of gel). Insert the sharp fork of pestle to the bottom of the tube and twist the tube back and forth repeatedly to reduce the gel to slurry. You can also punch the gel with the pestle repeatedly to reduce it to a fine slurry (it takes about 1-2 min, the pestle is reusable, for cleaning simply rinse with distilled water and dry it with paper towel). 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. Cap the tube and incubate at 94°C in a PCR machine for 5-10 min, vortex the tube briefly and continue to incubate for another 5-10 min. Longer incubation will increase protein yield. The capped tube can also be left at 4°C overnight.
4. Open the cap of the 200 µl tube and the cap of collection tube. Trim the caps off with a blade or a pair of scissors. Insert the opening end of 200 µl tube to the filter cartridge and centrifuge in a microfuge at top speed for 2 min. Discard the filter and save eluted protein in collection tube.

B. Nucleic acid extraction from agarose gel

1. Cut the band of interest out of the gel with a blade and trim away excessive gel.
2. Place gel piece(s) in the 200 µl tube provided. Add extraction powder to the tube (about 1/4 to 1/3 volume of the gel, see above).
3. Grind the gel with the pestle to reduce the gel to slurry as described above. Trim off caps of the 200 µl tube and collection tube. Insert the open end of the 200 µl tube to the filter cartridge and centrifuge at top speed for 1 min. Discard the filter and save eluted nucleic acid in collection tube.

Choice of elution buffers

The choice of elution buffer depends on how gel is stained and downstream applications. 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. If the gel is un-stained or negatively stained the protein can be eluted with ddH_2O or an elution buffer containing formic acid/ water/2-propanol (1:3:2 v/v/v).

Select References of Negative Staining:

1 Cohen, S. L., and Chait, B. T., (1997) Anal Biochem **247,** 274-267
2. Ortiz.,M. L., et al., (1992) FEBS Lett**. 296**, 300-304