Extract proteins from polyacrylamide gels

Introduction

Researchers often use sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as an analytical tool to assess protein purification. Because of its excellent ability to resolve individual components of complex mixtures, SDS-PAGE may be used not only for evaluating purity but also as an active step in the purification process. Protein purification by gel electrophoresis has been used in various applications, such as antigen preparation for antibody generation, and isolation of proteins for identification by N-terminal sequencing and mass spectrometry. In this Tech Tip, various methods of extraction (elution) of proteins from polyacrylamide gels are described.

The first step in purifying a protein from a polyacrylamide gel is to locate the electrophoresed protein of interest in the gel. There are two options for band identification: 1) staining an outermost lane of the gel and then aligning it with the unstained portion of the gel to determine which section of the unstained gel should be excised, and 2) staining the entire gel with a negative stain or other type of stain that can be reversed after excising the band. The second step in purifying electrophoresed protein from a polyacrylamide gel is to extract (elute) the protein from the gel matrix.

Identify and excise the band of interest

Option 1—stain reference lane of the gel

1. After electrophoresis, use a clean scalpel to cut off a section or strip (one or more outermost lanes) of the gel that includes the molecular weight marker and one lane of the protein sample, preferably adjacent to the lanes from which the protein band(s) will be excised. Place the strip in a tray for staining and place the rest of the gel on a glass plate. (Wrap the gel in plastic to prevent it from drying while staining the strip in step 2.)
2. Stain the strip using a convenient protein stain such as Invitrogen™ SimplyBlue™ SafeStain (Cat. No. LC6060) or the Thermo Scientific™ Pierce™ Silver Stain Kit (Cat. No. 24612). This strip will function as the “reference” gel strip.
3. Align the stained strip with the unstained gel portion, and cut out a section of the unstained gel that aligns with the stained protein of interest in the reference strip. Bands of gel just above and below the region presumed to contain the protein of interest may also be excised and processed. If desired, stain the entire remaining gel after excision of the bands to determine the accuracy of excision.
4. Proceed to “Elute protein from the gel matrix”.

Option 2—stain gel with a negative (and/or reversible) stain

Negative (reverse-image) staining involves staining portions of gel that do not contain proteins, thereby leaving the proteins both unstained and identifiable against the stained background. Alternatively, a positive stain that can be reversed (“erased” from the proteins) after excising the desired bands may also be used. The Thermo Scientific™ Pierce™ Zinc Reversible Stain Kit (Cat. No. 24582) features a negative and reversible protein stain for polyacrylamide gels. The stain produces an opaque white background and leaves protein bands as clear, unstained areas that are visible when the gel is held over a dark background.

thermo scientific

To use the Pierce zinc stain in the current application, first stain the gel according to the product instructions. Then excise the unstained bands of interest, and proceed directly to the protein elution steps. Alternatively, erase residual stain from the edges of the excised gel pieces by soaking them for 5–10 min in Tris-glycine buffer (25 mM Tris, 192 mM glycine, pH 8.0; same as Cat. No. 28380 prepared without methanol). **Note:** Although the Pierce zinc stain kit includes an eraser solution that erases the stain more quickly than the Tris-glycine buffer, it inhibits subsequent elution yields and is not recommended for this application.

Elute protein from the gel matrix

Option 1—passive elution of protein from polyacrylamide gel pieces

1. Place excised gel pieces in clean screw-cap culture or microcentrifuge tubes.
2. Add 0.5–1 mL of elution buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1 mM EDTA, pH 7.5) so that the gel pieces are completely immersed.
3. Crush the gel pieces using a clean pestle, and incubate on a rotary shaker at 30°C overnight.
4. Centrifuge at 5,000–10,000 x *g* for 10 min, and carefully pipet the supernatant into a new microcentrifuge tube. A portion of the supernatant may be tested for the presence of protein by subjecting it to SDS-PAGE.

Option 2—electroelution of protein from polyacrylamide gel pieces

In this technique, protein-containing gel pieces are placed in an electroelution chamber, where the proteins are eluted from the gel matrix into a buffer solution using an electrical field and captured against a dialysis membrane with an appropriate molecular weight cutoff. Several manufacturers of electrophoresis boxes and transfer cassettes offer compatible attachments designed for this application; contact the manufacturer of your gel electrophoresis apparatus for more information.

Ordering information

Product	Quantity	Cat. No.
SimplyBlue SafeStain	1 L	LC6060
Pierce Silver Stain Kit	1 L	24612
Pierce Silver Stain for Mass Spectrometry	1 L	24600
Pierce Zinc Reversible Stain Kit	1.5 L	24582
PageRuler Unstained Protein Ladder	2 x 250 µL	26614
BupH Tris-Glycine Buffer Packs	40 packs	28380
MemCode Reversible Protein Stain Kit for Nitrocellulose Membranes	1.5 L	24580

Find out more at **thermofisher.com/proteinstains**

ThermoFisher SCIENTIFIC