

Purify proteins from polyacrylamide gels

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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 for extraction (elution) of proteins from polyacrylamide gels are described.

The first step in purifying proteins from polyacrylamide gels is to locate the electrophoresed protein of interest in the gel. There are two options for band identification: 1) stain a side strip of the gel and then align it with the unstained portion of the gel to determine which section of the unstained gel should be excised and 2) stain 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 polyacrylamide gels is to extract (elute) the protein from the gel matrix.

Identify and Excise the Band of Interest

• Option 1: Stain Side Strips of the Gel

1. After gel electrophoresis, use a clean scalpel to cut off a strip on the right or left of the gel (to include the molecular weight marker lane and the first lane of protein sample). Place the strip in a tray for staining; 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 cut strip of gel using a convenient protein stain such as Thermo Scientific GelCode Blue Stain Reagent (Product No. 24590) or Pierce Silver Stain Kit (Product No. 24612). This strip will function as the “reference” gel strip.
3. Align the stained strip of gel with the unstained gel portion and cut out the band of 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 bands to determine the accuracy of excision.
4. Proceed to the “Elute the Protein from the Gel Matrix” section.

• 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 identified band may also be used. Pierce[®] Zinc Reversible Stain Kit (Product No. 24582) is both a negative and a 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.

To use the Zinc Stain in the current application, first stain the gel according to the product instructions. Once the unstained band of interest has been excised, 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 minutes in Tris-glycine buffer (25 mM Tris, 192 mM glycine, pH 8.0; same as Product No. 28380 prepared without methanol). Although the Zinc Stain Kit includes an Eraser solution that erases the stain more quickly than Tris-glycine buffer, it inhibits subsequent elution yields and is not recommended for this application.

Elute the Protein from the Gel Matrix

- **Option 1: Passive Elution of Proteins 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, and 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 in a rotary shaker at 30°C overnight.
4. Centrifuge at 5,000-10,000 × g for 10 minutes and carefully pipette supernatant into a new microcentrifuge tube. An aliquot of the supernatant may be tested for the presence of protein by subjecting it to SDS-PAGE.

- **Option 2: Electroelution of Proteins 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 cut off. 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.

Additional Information

- Tech Tip #50: Process stained polyacrylamide gel pieces for mass spectrometry
- Tech Tip #49: Acetone precipitation of proteins

Related Thermo Scientific Products

24590	GelCode™ Blue Stain Reagent
24602	Pierce Silver Stain Kit
24582	Pierce Zinc Reversible Stain Kit
26681	Pierce Blue Prestained Protein Molecular Weight Marker Mix
28380	BupH™ Tris-Glycine Transfer Buffer Packs
24580	MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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