Production of polyclonal antibodies against protein antigens purified by electroelution from SDS-polyacrylamide gel

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Method Article

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Abstract

Introduction

This protocol describes the purification of insoluble or moderately soluble proteins using an electro-separation system for elution from SDS-polyacrylamide gel and the use of the purified proteins for the production of polyclonal antibodies. The proteins to be purified must be highly expressed in bacterial cells and visible in polyacrylamide gels following Coomassie Blue staining. The gel should not be stained because non-stained proteins are electroelueted much faster than fixed and stained proteins and Coomassie Blue cannot be removed by electroelution.

Reagents

Liquid and solid LB medium with appropriate selection _E. coli_ plasmid expression vector carrying cDNA or cDNA fragment of interest Protease-deficient _E. coli_ strain 6X SDS-PAGE Loading Buffer \[0.35 M Tris-HCl, pH 6.8, 10.28% \(w/v) SDS, 36% \(v/v) glycerol, 0.6 M dithiothreitol, 0.012% \(w/v) bromophenol blue] 0.3% Coomassie Blue in 10% acetic acid 30% ethanol/10% acetic acid solution 15 mM ammonium bicarbonate solution

Equipment

S&S BIOTRAP device \(Schleicher and Schuell) BT1 and BT2 membranes \(Schleicher and Schuell) Amicon centrifugal filters \(Millipore) Incubator at 37°C with and without shaking

Procedure

1. Transform protease-deficient _E. coli_ strain \(e.g., BL21) with a plasmid expression vector carrying cDNA or cDNA fragment of interest. 2. Spread transformation on appropriate LB medium plate and incubate overnight at 37°C. 3. Inoculate 5 ml of LB medium with a single colony of _E. coli_ cells and incubate overnight at 37°C with shaking. 4. Inoculate 50 ml of LB medium in 250 ml E-flask with 500 µl of culture \(dilution 1:100) and grow at 37°C with shaking until the absorbance at 600 nm reaches 0.5-0.7. 5. Add the suitable inducer for the expression of the protein of interest according to the plasmid vector used \[e.g., 0.5 mM \(final concentration) IPTG] and incubate for an additional 2 h. 6. Centrifuge the cell culture at 7,700g for 10 min at 4°C, discard the supernatant, and wash the pellet with 1X Phosphate-Buffered Saline \(PBS). 7. Suspend the cell pellet by adding 50 µl of 1X PBS per ml of culture. 8. Sonicate on ice in short bursts to disrupt the cells until a partial clearing of the suspension is observed. 9. Add an appropriate aliquot of SDS-PAGE Loading Buffer to the cell homogenate and load 100-150 µl per well on a large \(e.g., 18 X 20 cm) SDS-polyacrylamide gel as in **Fig. 1a**. 10. Run gel according to standard methods until maximum separation of the protein of interest is obtained. 11. Cut two vertical strips on the sides of the gel as ilustrated in **Fig. 1b** and stain them in 0.3% Coomassie Blue/10% acetic acid for 5 min. Leave the rest of the gel sitting in running buffer. 12. Discolour strips in 30% ethanol/10% acetic acid

solution, realign them to the gel sides, and cut a long horizontal slice at the level of the band of interest as shown in **Fig. 1c**. 13. Cut the long strip into 1-cm pieces and place them vertically into the mini elution chamber of the S&S BIOTRAP device \(Schleicher and Schuell) \(**Fig. 2**). A number of gel pieces sufficient to fill the elution chamber can be inserted to keep them upright \(if you cannot proceed immediately to electroelution, the strip pieces can be stored at -20°C for few days). 14. Perform electroelution as specified by the manufacturer at 200 V for 4 h using 15 mM ammonium bicarbonate as the elution buffer. 15. After elution is completed, remove eluate from trap chamber using a Pasteur pipette and store it at -80°C until use. 16. Load an aliquot of elution product on mini SDS-polyacrylamide gel along with bovine serum albumin \(BSA) of known concentration to evaluate quantity and purity of the purified protein \(**Fig. 3**). 17. If necessary, purified proteins may be concentrated to the required concentration using suitable centrifugal filters \(e.g., Millipore Amicon) and/or lyophilized and stored at -80°C. 18. Finally, purified proteins in ammonium bicarbonate buffer or lyophilized may be inoculated in rabbits using standard protocols to produce polyclonal antibodies.

Critical Steps

Gel strips usually undergo size reduction during the staining and discoloration steps, making it difficult to accurately realign them to the rest of the gel. In this case, it is important to realign as accurately as possible the bands of the molecular weight marker at the level of the protein of interest.

Figures

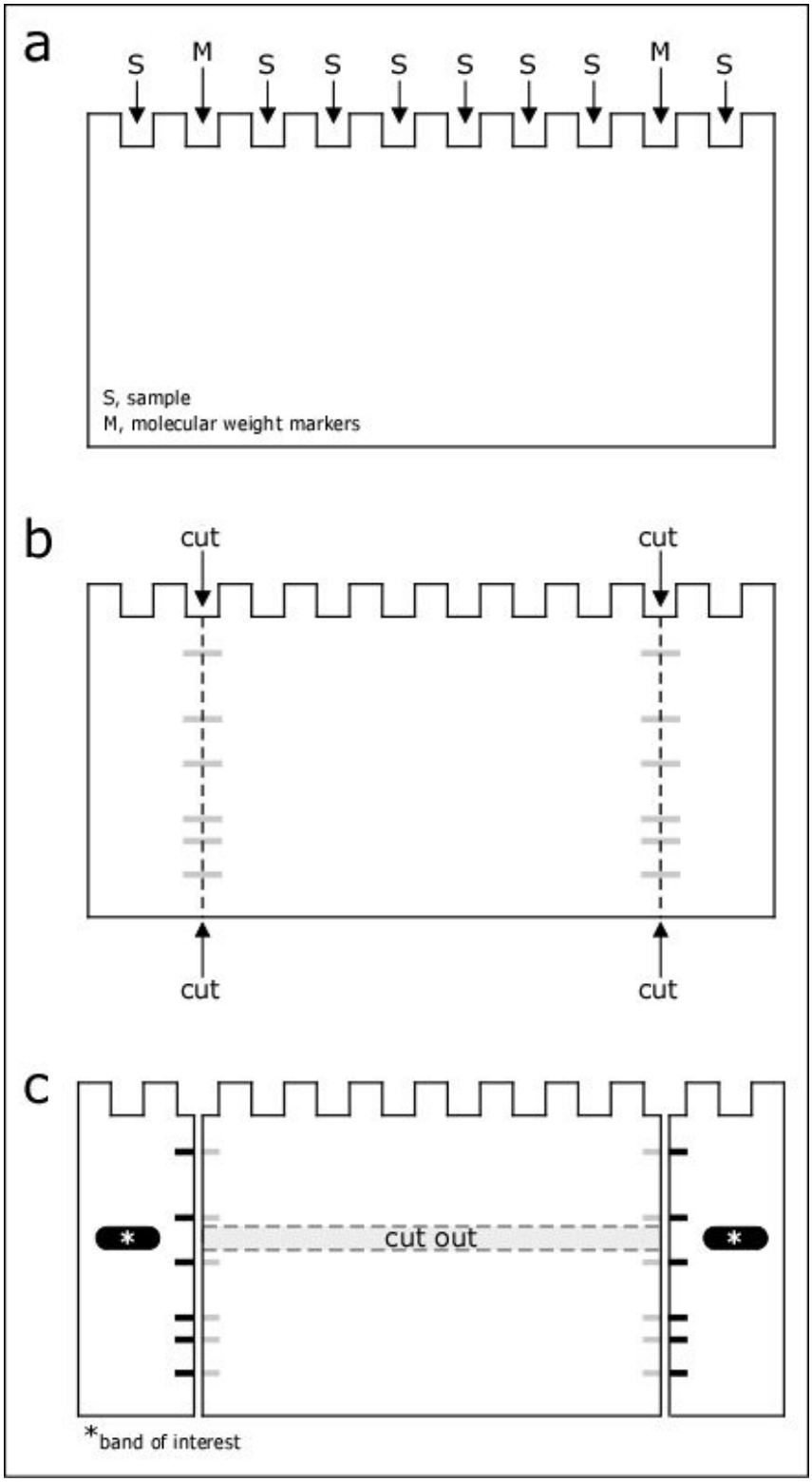

Figure 1

SDS-PAGE separation of proteins and slicing out of the protein of interest (*a*) Run gel according to standard methods until maximum separation of the protein of interest is obtained. (*b*) Cut two vertical strips on the sides of the gel. (*c*) Stain strips in 0.3% Coomassie Blue/10% acetic acid solution for 5 min, while the remainder of the gel is retained in gel running buffer. Realign strips to the gel sides and cut a long horizontal slice at the level of the band of interest (*).

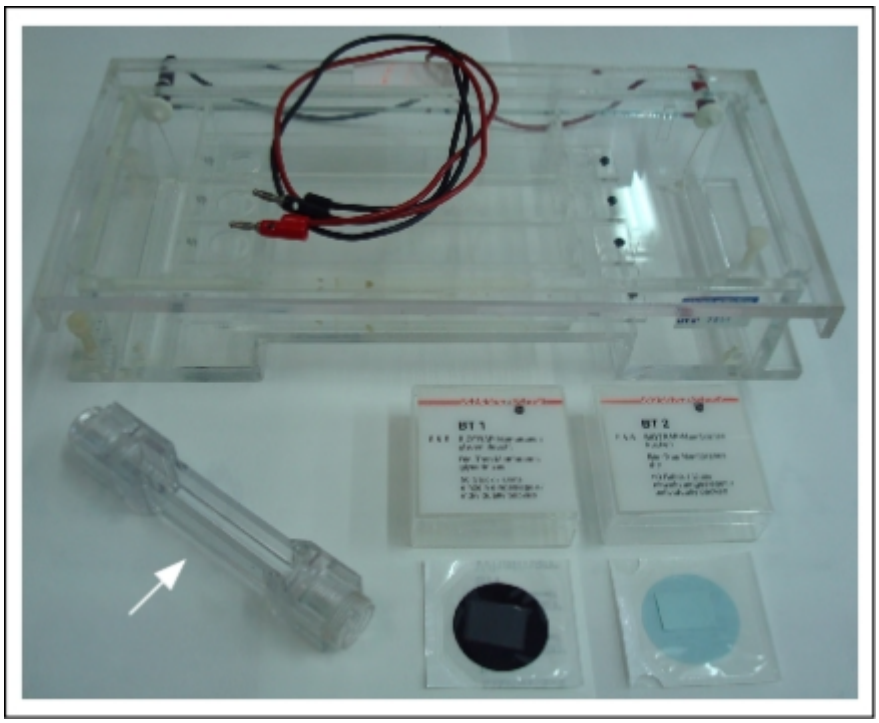

Figure 2

Electroelution apparatus Cut the long strip in Fig. 1c into 1-cm pieces and place them vertically into the mini elution chamber (arrow) of the S&S BIOTRAP device (Schleicher and Schuell). A number of gel pieces sufficient to fill the elution chamber can be inserted to keep them upright.

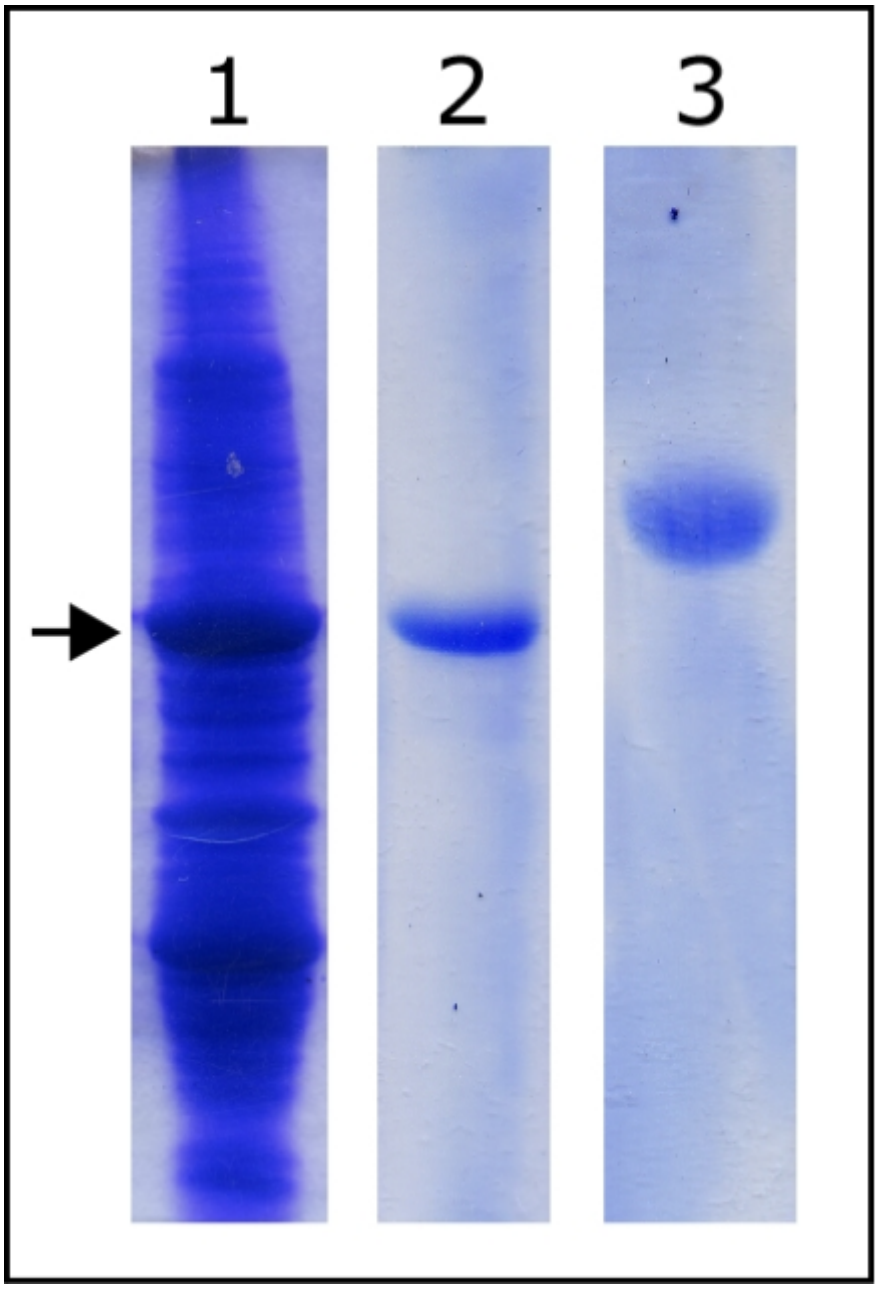

Figure 3

SDS-PAGE of electroeluted protein Lane 1: Bacterial cell total protein extract from which the protein of interest (arrow) is electroeluted. Lane 2: elution product. Lane 3: Bovine serum albumin (BSA) of known amount (10 µg) to quantitate the purified protein.