INSTRUCTIONS

Immobilized *E. coli* Lysate

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44938

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Number	Description
44938	**Immobilized *E. coli* Lysate,** 5 ml settled gel, contains bacterial lysate from *E. coli* BMH 71-18, immobilized on 6% cross-linked beaded agarose supplied as a 33% slurry (~15 ml total volume) in Tris buffered saline Loading: 1.5 mg *E. coli* protein/ml of gel **Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

Immobilized *E. coli* Lysate provides a simple and efficient method of removing *E. coli*-reactive proteins from antibody preparations. Partially purified bacterial proteins from a suspension of *E. coli* cells (strain BMH 71-18) are immobilized onto agarose by a proprietary method that yields an efficient affinity support. When serum is added to the support, *E. coli* binding proteins bind, and the purified antibody preparation flows through.

Crude antisera and ascites fluid containing an expressed protein of interest often contains antibodies and other proteins that bind to *E. coli* proteins. When using antibodies to screen a recombinant DNA library, these *E. coli* binding proteins can cause high background resulting in false positive plaques. False positives especially are likely to occur if the titer or affinity of the *E. coli* binding proteins is greater than the antibody to the protein of interest. Optimizing the dilution of primary antibody for screening may eliminate some of the nonspecific background; however, using Immobilized *E. coli* Lysate to pre-adsorb the *E. coli* binding proteins can reduce or eliminate nonspecific binding and ease the screening process.

Procedure for Removing *E. coli*-reactive Proteins Using a Gravity-flow Column

Note: The Immobilized *E. coli* Lysate protocol may be modified for use in a spin-column format.

A. Additional Materials Required

- Tris Buffered Saline (TBS): 25 mM Tris, 150 mM NaCl; pH 7.2-7.6 (BupH™ Tris Buffered Saline Packs Product No. 28379)
- Regeneration Buffer: 0.1 M glycine, pH 2.8
- Disposable column capable of containing at least 1 ml gel-bed volume such as the Disposable Polypropylene Columns (Product No. 29922) or the Disposable Column Trial Pack (Product No.29925) that contains two each of three column sizes.

  Note: For spin-column formats, use Handee™ Mini-Spin Columns and Accessories (Product No. 69705).

B. Protocol

1. Carefully pack the column with the desired amount of Immobilized *E. coli* Lysate slurry. Follow the packing instructions provided with the columns.
2. Before use equilibrate Immobilized *E. coli* Lysate column to room temperature.
3. To avoid air bubbles being drawn into gel, open packed columns by first removing the top cap and then the bottom cap from the column. Decant and properly discard storage solution.
4. Equilibrate column with at least two column volumes of TBS.

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5. Add sample to the column.
6. After the entire sample has entered the gel, add 100-1,000 µl of TBS to the column and begin collecting fractions.

   Note: The spin-column format may require incubation for 15-90 minutes at room temperature for maximum efficiency.
7. Monitor each fraction by measuring the absorbance at 280 nm. Continue adding TBS until the absorbance reaches baseline. Pool protein fractions of interest.
8. Wash the column with at least five times the column gel-bed volume of Regeneration Buffer. The column can be reused at least four times without loss in adsorbing capacity.
9. After regeneration, immediately wash the column with at least five times the column gel-bed volume of TBS containing 0.02% sodium azide or other suitable storage solution.
10. Store the column upright at 4°C.

    Note: Dedicate each Immobilized *E. coli* Lysate column to one type of antiserum to prevent cross contamination.

Related Pierce Products

89875	**ProteoSeek™ Albumin/IgG Removal Kit**
89876	**ProteoSeek™ Antibody-Based Albumin/IgG Removal Kit**
34080	**SuperSignal® West Pico Chemiluminescent Substrate,** 500 ml
34075	**SuperSignal® West Dura Extended Duration Substrate,** 100 ml
34095	**SuperSignal® West Femto Maximum Sensitivity Substrate,** 100 ml
21059	**Restore™ Western Blot Stripping Buffer,** 500 ml
34090	**CL-XPosure™ Film (5" x 7"),** 100 sheets
34091	**CL-XPosure™ Film (8"x 10"),** 100 sheets
21065	**Erase-It® Background Eliminator Kit,** for eliminating background from X-ray film
25200-25244	**Precise™ Protein Gels** (see catalog or web site for a complete listing)

Product Reference

Fox, D. and Smulian, G. (1999). Mitogen-activated protein kinase Mkp1 of Pneumocystis carinii complements the slt2Δ defect in the cell integrity pathway of *Saccharomyces cerevisiae*. *Mol. Microbiol.* **34(3):**451-62.

General References

Current Protocols in Molecular Biology, p 6.0.3.

Berger, S.L. and Kimmel, A.R. eds. (1987) Guide to Molecular Cloning Techniques. *Meth. Enzymol*. **152:**467-469.

Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd edition, p. 12.2-12.3.

*SuperSignal® Technology is protected by U.S. Patent # 6,432,662

The most current versions of all product instructions are available at *www.piercenet.com*. For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.

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