Western Blot of Protein Expressed by Yeast

Protein Preparation

1. Inoculate 4ml of DOB-Trp media with a single yeast colony. Shake culture for 3-4 days at 30oC.
2. Transfer 1.0ml of this culture to 1.5ml microcentrifuge tubes and pellet yeast cells with quick spin.
3. Aspirate supernatant and resuspend yeast cells in 100ul of loading buffer.
4. Measure out 25ul of glass beads in microcentrifuge tube.
5. Transfer resuspended yeast cells to tube containing glass beads. Vortex cells/beads for 1-2 minutes.
6. Boil yeast cells/beads for 10 minutes.
7. Quick spin busted cells.

SDS-PAGE

8. Run 25ul of the supernatant on a 5% stacking/8% resolving acrylamide gel. Use 7ul of a protein standard. Run gel at 100V until loading dye is at the bottom edge of the gel.

Transfer of Proteins

9. Cut 4 pieces of white paper and 2 pieces of transfer paper.
10. Fill pyrex dish half full with western transfer buffer. Place transfer frame in buffer.
11. The components of the transfer were placed on the cathode side of the transfer frame in the following order: porous pad, one piece of white paper, the trimmed gel containing the proteins to be transferred, one piece of transfer paper, one piece of white paper, and porous pad.
12. Close frame and place in the transfer apparatus. (A container of frozen water may be placed in apparatus, depending on the size of the transfer)
13. Hook up electrodes and set power supply at 30V to transfer the proteins overnight.
14. Disassemble transfer apparatus and remove transfer paper. Trim paper to size of gel.
15. Fill container half full with %5 blocking milk solution. Add transfer paper and shake at 55 RPM for 1 hour.
16. Discard milk solution. Cover transfer paper with TBST and shake at 55 RPM for 30 minutes.
17. Discard TBST. Cover transfer paper with fresh TBST and shake at 55 RPM for 1 hour.
18. Discard TBST. Cover transfer paper with the primary antibody and shake at 55 RPM for 1 hour.

19. Remove antibody (save and reuse). Wash the transfer paper 4 times with TBST: 2 times for 15 minutes; 2 times for 10 minutes. Use fresh TBST for each wash and shake at 55 RPM during each wash.
20. Add secondary antibody to transfer paper and shake at 55 RPM for 1 hour. Remove secondary antibody (save and reuse). Wash transfer paper four times as before.
21. Wet surface of transfer paper with 1.5 ml of each of the two ECL reagents. Let reagents set for one minute.
22. Discard reagents. Dry out transfer paper slightly and place on a firm piece of paper. Wrap papers in saran wrap. Place in cassette and expose to film in dark room for 3 minutes. Processes film.