Yeast Protein Extraction Protocol

This protocol is designed to extract total yeast proteins and to detect MSH2 by western blot

Alkaline Lysis method:

Transformed Yeast cells were grown in 5 mL selective medium (SD–his –trp) overnight to log phase (~ 36h) OD600 ~0.5-1.0.

1. Take 2.5 mL of cultured cells in a 15mL centrifuge tube and spin them down at 4000 rpm for 5 mins.
2. Discard the medium to a dish provided in the sink.
3. Re-suspend cells in 1 mL autoclaved Di water and transfer the re-suspended cells into a fresh microcentrifuge tube
4. Centrifuged the cells again at 13000 × g (max speed for microcentrifuge) for 1 min.
5. Re-suspend the cell pellet in 100 μL alkaline lysis buffer provided (0.1M NaOH, 0.05M EDTA, 2% SDS and 2% BME)
6. Heat mixture at 90oC for 10 min
7. Neutralize mixture by adding 4 μL of 4M acetic acid
8. Heat again at 90oC for 10 min
9. Spin the samples again at max speed (14000 rpm) for 5 min
10. Transfer the clarified supernatant into a fresh tube (this is your protein extract)
11. Prepare samples for gel: add 20 μl of protein extract to 20 μL of sample buffer (62.5 mM TRIS pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue and 5% BME). If needed sample can be stored at -20°C.
12. Load 40 μL of prepared sample onto the SDS-PAGE gel (each sample well of a gel can contain 50 μL).