

Immunoprecipitation of yeast proteins in denaturing conditions for analysis of ubiquitylation - METHOD 2

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INTRODUCTION

The aim of this experiment is to immunoprecipitate proteins from yeast in denaturing conditions. After blotting of the immunoprecipitate with anti-Ubiquitin antibodies (ex. clone P4D1), this allows to verify the ubiquitylation status of a given protein. This protocol is adapted from Kiel et al. 2005.

MATERIALS

Resuspension buffer

- 1% SDS, 0.1 M NaOH

Urea cracking buffer

- 50 mM Tris-HCl pH 7.5
- 6M Urea, 1% SDS

Tween 20-IP buffer

- 50 mM Tris-HCl pH 7.5
- 150 mM NaCl
- 0.5% Tween-20
- 0.1 mM EDTA

Tween 20-Urea buffer

- 100 mM Tris-HCl pH 7.5
- 200 mM NaCl
- 2M Urea
- 0.5% Tween-20

IP sample buffer

- 125 mM Tris-HCl pH 6.8
- 20% glycerol
- 6% SDS
- 10% b-ME
- 0.1% bromphenol blue

METHODS

1. Precipitate 3 ODs of log-phase yeasts with 10% TCA (final concentration)
2. Incubate at least 1h on ice.
3. Spin down.

4. Wash twice with cold (-20°C) 80% acetone
5. Dry pellet at room temperature.

Note: For a crude extract, resuspend in 80 µL of resuspension buffer, and after 30 min at 4°C, add 20 µL of 5x SDS buffer, then boil for 5 min at 100°C

6. Add 100 µL urea cracking buffer and incubate for 10 min at 65°C.
7. Add 1 mL Tween-20 IP buffer containing 0.5% BSA. Pellet insoluble material (10 min, 13,000 g).
8. Add antibody and rotate mixture for 1h at 4°C.
9. Add 75 µL Sepharose-ProteinG (GammaBind, GE Healthcare) and rotate for 1h at 4°C.
10. Wash twice with Tween-20 IP buffer and once with Tween-20 Urea buffer
11. Elute immunoprecipitate by boiling the beads in 50 µL IP sample buffer. Analyze by SDS-PAGE and immunoblotting. Anti-ubiquitin can be used to see ubiquitin conjugates (Anti-Ub P4D1, Anti-Ub(P4D1)-HRP, FK1/FK2, monoclonal or polyclonal depending on the antibody used for the immunoprecipitation). His-tagged, HA-tagged or myc-tagged ubiquitins and their corresponding antibodies are also good alternatives.

BIBLIOGRAPHY

Kiel, J.A., K. Emrich, H.E. Meyer, and W.H. Kunau. 2005. Ubiquitination of the Peroxisomal Targeting Signal Type 1 Receptor, Pex5p, Suggests the Presence of a Quality Control Mechanism during Peroxisomal Matrix Protein Import. *J Biol Chem.* 280:1921-30.