Co-Immunoprecipitation assay for HA-tagged proteins from yeast

Carmen Bano (✉ bano@uv.es)

Cell Cycle, Universitat València

Juan Carlos Igual (✉ jcigual@uv.es)

Cell Cycle, Universitat València

Inma Quilis (✉ quibain@uv.es)

Cell Cycle, Universitat València

Method Article

Keywords: Co-Immunoprecipitation HA-tagged proteins yeast

Posted Date: January 29th, 2019

DOI: <https://doi.org/10.1038/protex.2019.004>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Co-Immunoprecipitation assay for tagged proteins using yeast cells

Reagents

-Lysis buffer: 50 mM Tris-HCl pH 8, 250 mM NaCl, 5 mM EDTA, 0,1% (v/v) Triton X-100, and Complete Mini protease inhibitor (Roche Diagnostics) -Dynabeads Protein G (10004D, Invitrogen) - HA-probe (F-7) antibody (SC-7392, Santa Cruz Biotechnology Inc.) - PBS : 150 mM NaCl, 40 mM Na₂PO₄, 10 mM NaH₂PO₄ - Tween 20 (P7949-100ML, SIGMA ALDRICH) - 2x SDS-PAGE sample solvent: 4% SDS, 20% glycerol, 0.02% bromophenol blue, 0.1 M DTT, 0.125 M Tris-HCl pH 7.5

Procedure

Sample collection and protein extract 1. Collect approximately 5×10^8 cells expressing either untagged or HA-epitope tagged versions of protein to immunoprecipitate from exponentially growing yeast cultures. 2. Collect the cells by centrifugation at 4000xg and wash them once with H₂O. 3. Resuspend the cell pellet in 100 μ L of Lysis buffer. 4. Lyse the cells by vigorous shaking with glass beads for 3 min at 4°C. 5. Clarify the sample at 13400xg for 5 min at 4°C. 6. Keep 10 μ L from the supernatant as a control of whole-cell extract (Input). Dynabeads Protein G preparation 7. Wash twice 50 μ L of Dynabeads with PBS containing Tween 0.02%. 8. Incubate for 30 min at room temperature the dried beads with 10 μ L of HA-probe (F-7) antibody (Santa Cruz Biotechnology Inc) supplemented with 190 μ L PBS-Tween 0,02%. 9. Wash the unbound antibody twice with PBS-Tween 0,02%. Immunoprecipitation 10. Incubate the remaining volume of the whole cell extract with Dynabeads-antibody with orbital rotation for 20 min at room temperature. 11. Keep 10 μ L from the supernatant as a control of unbound protein. 12. Wash the beads 5 times with PBS-Tween 0,02%. 13. Elute the immunoprecipitated proteins by boiling the beads for 5 min in 2x SDS-PAGE sample solvent and analyze by SDS-polyacrylamide gel electrophoresis followed by Western blot analysis.