Co-Immunoprecipitation assay for HA-tagged proteins from yeast

CURRENT STATUS: POSTED

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DOI:
10.1038/protex.2019.004

SUBJECT AREAS
Biochemistry *Cell biology*

KEYWORDS
Co-Immunoprecipitation HA-tagged proteins yeast

Abstract

Co-Immunoprecipitation sassy 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 × 108 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 µL 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 4ºC.
6. Keep 10 µl from the supernatant as a control of whole-cell extract (Input).

Dynabeads Protein G preparation

7. Wash twice 50µL of Dynabeads with PBS containing Tween 0.02%.
8. Incubate for 30 min at room temperature the dried beads with 10µL of HA-probe (F-7) antibody (Santa Cruz Biotechnology Inc) supplemented with 190µL 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 µL 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.