Immunoprecipitation of Soluble Proteins from Yeast Cell Extracts

Solutions

PIPES/KOH buffer

- 0.035 g Na-azide
- 28 ml dH_2O
- 7 ml 0.5 M PIPES/KOH pH 9.4
- 350 µl 1M DTT (fresh)

Makes 35 ml = 100 mM PIPES/KOH pH 9.4, 10 mM DTT, 0.1% Na-azide

Kpi/sorbitol buffer

- 22.4 ml dH_2O
- 1.4 ml 1M K_2HPO_4
- 347 µl 1M KH_2PO_4
- 10.5 ml 2 M sorbitol
- 350 µl 1 M DTT (fresh)

Makes 35 ml = 50 mM KPi pH 7.4, 0.6 M sorbitol, 10 mM DTT

Spheroblast wash buffer

- 25 ml dH_2O
- 1.75 ml 1M HEPES/KOH pH 7.5
- 1166 µl 3 M KCl
- 87.5 µl 1 M $MgCl_2$
- 7 ml 2 M sorbitol

Makes 35 ml = 50 mM HEPES/KOH pH 7.5, 100 mM KCl, 2.5 mM $MgCl_2$, 0.4 M sorbitol

Protease inhibitors

- leupeptine (1000X): 3 mg/1.5 ml H_2O
- benzamidine(1000X): 0.469 g/1.5 ml H_2O
- aprotinin (1000X): 3 mg/1.5 ml H_2O
- bacitracin (1000X): 0.3 g/1.5 ml H_2O
- pepstatin (1000X): 3 mg/1.5 ml MeOH
- PMSF (250X): 0.436g/10 ml anhydrous EtOH

Zymolase

- 40 mg zymolase T-100 (ICN Biomedicals)
- 2ml 1M sorbitol

EB buffer

- 45.8 ml dH_2O
- 2.5 ml HEPES/KOH 1 M, pH 7.5
- 1665 µl KCl 3 M
- 125 µl $MgCl_2$ 1M
- 50 µl DTT 1M
- 200 µl leupep (1000X)
- 200 µl benzamid (1000X)
- 200 µl aprot (1000X)
- 200 µl bacitrac (1000X)
- 200 µl pepst A (1000X)
- 100 µl NaF 1M
- 5 protease inhibitor tablets (EDTA free, Roche)
- PMSF (250X), add freshly

Makes 50 ml = 50 mM HEPES/KOH pH 7.5, 100 mM KCl, 2.5 mM $MgCl_2$

EBX buffer

- 47 ml EB buffer
- 1175 µl 10% Triton X-100

Makes 47 ml = 50 mM HEPES/KOH pH 7.5, 100 mM KCl, 2.5 mM $MgCl_2$, 0.25% Triton X-100

EBX-S buffer

- 2.9 ml dH_2O
- 250 µl 10% Triton X-100
- 6 ml sucrose 50%
- 332 µl KCl 3M
- 500 µl HEPES/KOH 1 M, pH 7.5
- 25 µl $MgCl_2$ 1 M
- 10 µl DTT 1 M
- 40 µl leupep (1000X)
- 40 µl benzamid (1000X)
- 40 µl aprot (1000X)
- 40 µl bacitrac (1000X)
- 40 µl pepst A (1000X)
- 20 µl NaF 1M
- 2 protease inhibitor tablets (EDTA free, Roche)
- PMSF (250X), add freshly

Makes = 50 mM HEPES/KOH pH 7.5, 100 mM KCl, 2.5 mM $MgCl_2$, 0.25% Triton X-100, 30% sucrose

Procedure

1. Grow 250 ml of yeast culture until OD = 0.5.
2. Harvest cells (3000 rpm, 10 min, 4° C).
3. Resuspend in 5 ml PIPES/KOH buffer.
4. Incubate 10 min at room temperature.
5. Spin for 3 min at 2000 rpm, decant supernatant.
6. Resuspend in 5 ml Kpi/sorbitol buffer.
7. Take 10 µl, dilute in 990 µl dH_2O in cuvette and measure OD (600 nm).
8. Add 10 µl zymolase T-100
9. Incubate at 37° C for 10 min in waterbath.
10. Take 10 µl, dilute in 990 µl dH_2O in cuvette and measure OD (600 nm) after 1 min (value should be 10% of the value before zymolase treatment).
11. Spin for 5 min at 800 rpm.
12. Move to COLD ROOM (and keep everything on ICE!).
13. Wash cells in 5 ml ice-cold spheroblast wash buffer.
14. Spin for 5 min at 800 rpm, aspirate the supernatant (about 450 µl cell pellet remains).
15. Resuspend in 450 µl EB buffer (gives 900 µl whole cell extract) and transfer to 1.5 ml Eppendorf.
16. Add 20 µl 10 % Triton X-100, vortex and leave on ice for 3 min.
17. In the meantime, prepare 900 µl EBX-S in a 2 ml Eppendorf.
18. Slowly lay 900 µl of whole cell extract onto EBX-S.
19. Spin for 10 min at 12000 rpm.
20. Take off yellow supernatant above the sucrose cushion (= extract of soluble proteins).
21. Pre-clear supernatant with 100 µl protein A-sepharose (Amersham, equilibrated overnight in EBX buffer) for 30 min on a rotating wheel.
22. Spin for 1 min at 800 rpm.
23. Transfer supernatant into 1.5 ml Eppendorf and add 2 µl 12CA5 (anti-HA) or 9E10 (anti-Myc) antibody.
24. Leave on ice for 1 h.

25. Add 20 µl protein A-sepharose.
26. Allow binding reaction for 30 min on rotating wheel.
27. Wash protein A-sepharose 8 times with 1 ml EBX.
28. Add 40 µl 2X sample solution.
29. Vortex and then boil 5 min at 95° C.
30. Spin for 1 min at 13000 rpm.
31. Transfer supernatent into new 1.5 ml Eppendorf.
32. Load 20 µl on gel for silver staining.