

Protein Extraction

Adapted from Vincent 08/21/02

1. Harvest log-phase cells from 10-50ml culture at O.D. ~0.5 in Falcon tubes. Wash in cold PBS, pellet, and you can freeze them at -80°C.
2. Thaw pellets and resuspend in cold 0.5ml TE. Transfer to spin-cap tubes. Pellet and vacuum supernatant.
3. Prepare TBT buffer with inhibitors (0.4ml/sample) and without inhibitors 3ml/sample) for washing step.
4. Add 2 scoops glass beads (400ug) and 250µl TBT buffer. FastPrep in setting 5, x2 for 60 seconds each.
5. Spin 10 minutes at 10,000rpm at 4°C and transfer 100ul supernatant to fresh microfuge tubes.
6. Add 100ul of 2x sample buffer=total 200ul
7. Boil for 3 minutes

Co-IP (for example HA tagged protein)

1. Harvest log-phase cells from 10-50ml culture at O.D. ~0.5 in Falcon tubes. Wash in cold PBS, pellet, and you can freeze them at -80°C.
2. Thaw pellets and resuspend in cold 0.5ml TE. Transfer to spin-cap tubes. Pellet and vacuum supernatant.
3. Prepare TBT buffer with inhibitors (0.4ml/sample) and without inhibitors (3ml/sample) for washing step.
4. Add 2 scoops glass beads (400ug) and 400µl TBT buffer. FastPrep in setting 5, x2 for 60 seconds each.
5. Spin 10 minutes at 10,000rpm at 4°C and transfer supernatant to fresh microfuge tubes.
6. re-equilibrate resin as follows:
 - Aliquot 30µl monoclonal anti-HA agarose resin (Sigma, A2095-1ML) into fresh microfuge tubes
 - Add 1ml 1xPBS and spin
 - Pellet and add 200µl TBT buffer
7. Add pooled cell extract (about 200-300ul) to pre-equilibrated resin and incubate on nutator at 4°C for 1-2 hr.
8. Collect resin by spinning at 3,000 rpm at 4°C for 30 sec.
9. Wash x3 with 1ml TBT (without inhibitors) (do not vortex! be gentle.).
10. After final wash, remove supernatant and add 100µl 1x sample buffer. Heat at 95°C for 5 min, quick spin, and freeze.

Reagents

TBT-DNase buffer (500 ml, 100mM AcOK, 20mM KHEPES pH 7.4, 2mM MgCl₂, 0.1% Tween 20, 1mM DTT, 0.002% DNase, Protease inhibitors)

4.9g	Potassium acetate (AcOK)
20ml	0.5M KHEPES pH 7.4 (Sigma H0527)
1ml	1M MgCl ₂
350 ml	Autoclaved dH ₂ O
0.1%,	Tween 20 (Fisher BP337)

Fresh before use

1x PMSF, 1x Aprotinin, 1x Leu-pep/p, 1x Nappi, 0.5x PhosSTOP
(PMSF Sigma P7626, Aprotinin Sigma A6279, Leupeptin Sigma
L2884, Pepstatin Sigma P5318, Nappi Sigma 221368,
PhosSTOP Roche 4906845001)