TAP-Protein prep for *in vitro* kinase assay

5/28-2015 (modified by Ikui)

Cultures:

1. Grow O/N culture to O.D. (0.02-0.05) in 25 ml Sc-ura + 3% raffinose. Grow O/N in 30°C
2. Dilute O/N culture to O.D (0.3-0.6) in 100 ml Sc-ura + 3% Raffinose. Grow to O.D. (0.5-0.8).
    a. Pre-induction samples: take 5 ml pellet, and freeze in -80°C
3. Add 3% galactose, grow to O.D 1.0. Take 5ml post-induction samples.
4. Spin samples in 250 ml bottles at 3000 rpm, 4°C for 10 minutes
5. Transfer pellets and wash with 10 ml cold H_2O into 50 ml conical tubes
6. Spin at 3500rpm, 4°C for 6 minutes.
7. Pellet and freeze in -80°C

Lysis and IP:

1. Pre-induction sample extraction:
    a. Transfer samples (step 3a above) to spin cap tubes with 1ml TE and pellet.
    b. Add 2 scoops glass beads and 300µl 1x SDS onto pellet. Fast prep twice.
    c. Heat at 95°C for 5 min. Spin for 2 min.
2. Post-induction sample (step 4 above) extraction: Same as step 1.
3. Resuspend pellets (step 8 above) in 300ul TAP lysis buffer (+ fresh inhibitors)
    a. Add 2 scoops of glass beads into cap tubes per sample
    b. Fast prep (setting 6) for 60 seconds **twice**. (wait 5 minutes between the shake) Spin, then pool 6 supernatants in a tube.
    c. Collect 20µl samples for western + add 20µl 2x sample buffer
4. Spin down lysates for 1 hr in 10,000 rpm, 4°C. Collect 20µl from supernatant for western
5. Equilibrate rabbit IgG agarose beads (Sigma) and add samples:
    a. Prepare 1ml TAP Core + fresh inhibitors per sample
    b. Equilibrate 40µl beads in 1ml TAP core buffer for each sample in spin cap tubes
        - Spin at 3000 rpm, discard supernatant
    c. Add supernatant from samples into beads
    d. Put tubes on rocker in 4°C for 2 hours
    e. Spin down beads for 5 minutes at 1500 rpm, 4°C. Take 20µl sample from supernatant for western
    f. Wash with 1.5 ml TAP CORE buffer, and spin for 15 min at 4°C. Repeat 5 times
6. Cleave off TAP bound to beads using TEV:
    a. Wash with 1ml TEV cleavage buffer, spin for 10 min, RT. Remove supernatant
    b. Add 30µl TEV buffer, 2µl TEV protease. Incubate in 30°C for 30 minutes
    c. Spin down. Aliquot 10µl from each into eppendorf tubes. Take 1µl sample for western. Add 10µl 1x SDS Buffer
    d. Store purified proteins in -80°C.
    e. (if you use 3C protease from Clonetech, incuabte at 4 degrees ON)

7. wash beads with 1ml H2O, and add 20ul 1x sample buffer to tube with beads for western.

Reagents:

TAP lysis Buffer (200 ml stock, 50mM HEPES 7.4, 300mM NaCl, 0.1% NP40, 50mM Naf, 80mM BGP)

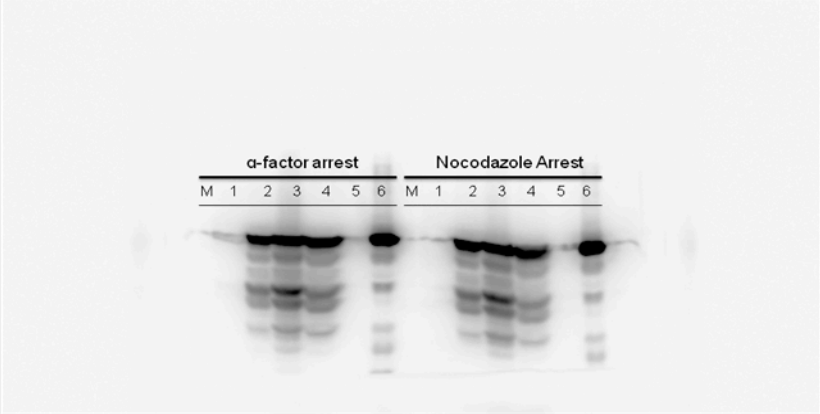
20ml	0.5M HEPES pH 7.4 (Sigma H3375)
12 ml	5M NaCl
200µl	100% NP40 (Fisher NC9375914)
20ml	500mM NaF (Sigma S7920)
16ml	1M BGP (Sigma G9422)
131.8ml	Autoclaved dH_2O
Fresh before use	Protease inhibitors, Phosphatase Inhibitors, PMSF, Aprotinin (PMSF Sigma P7626, Aprotinin Sigma A6279)

TAP Core (500 ml, 50mM HEPES 7.4, 150mM NaCl, 0.1% NP40, 50mM Naf, 80mM BGP)

50ml	0.5M HEPES pH 7.4 (Sigma H3375)
15ml	5M NaCl
500µl	100% NP40 (Fisher NC9375914)
50ml	500mM NaF (Sigma S7920)
40ml	1M BGP (Sigma G9422)
344.5ml	Autoclaved dH_2O
Fresh before use	Aprotinin, PMSF (PMSF: Sigma P7626, Aprotinin: Sigma A6279)

TEV cleavage Buffer (200ml)

20ml	500mM HEPES pH 8.0 (Sigma H3375)
6ml	5M NaCl
200µl	100% NP40 (Fisher NC9375914)
200µl	500mM EDTA (Fisher BP118)
20ml	100% Glycerol (Fisher G33)
153.4ml	Autoclaved dH_2O
200ul (add before use)	1M DTT (Sigma D0632)



DOM 638 – Mck1-TAP IP
Probed with anti-PrA-HRP, 1:5000, 1 hr
Exposure: 1 min, ECL regular
Mck1-TAP expected size: 62 kDa
07/16/2013