CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

This protocol generally yields 40 ml of crude (~70% pure) δ90 protein at ~0.5 mg/ml, which is active for induction of mitosis at a dilution of 1:20.

Procedure

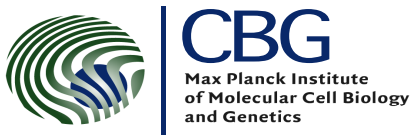
1. Grow 1 l pT7406 in BL21(DE3)pLysS in LB + amp + chloramphenicol to OD 0.4 at 37 C.
2. Induce with 100 µg/ml IPTG for 1 hour.
3. Pellet cells at 4 C., resuspend and wash pellet with 100 ml 0.9% NaCl, pellet.
4. Resuspend pellet in 25 ml T10N50E1 pH 8.0. (Tris, NaCl, EDTA).
5. Add DTT to
    - 5mM
    - chymostatin, pepstatin and leupeptin to 10µg/ml
    - NP-40 to 0.5%.

    incubate 15 min on ice. Should turn snotty. Sonicate 2' on ice.
6. Pellet in sorvall SS-34 or equivalent 15 min 12 k rpm.
7. Wash pellet in 10 ml T10N500E1 pH 8.0. Spin as above.
8. Treat pellet with DNase and RNase as described in Antibodies (Harlow and Lane). Be sure to add MgCl2 for the DNase.
9. Resuspend pellet in 20 ml 8M urea made in T10N50E1DTT5 pH 8.0. There will be some translucent material which will not resuspend.
10. Slowly dilute with Buffer A and spin 5 min 12 krpm HB-4.
11. dialyse against 1 liter buffer A, three changes at least 4 hrs each.
12. assay by morphology or activation of H1 kinase activity in interphase Xenopus extracts. I find that this type of ?90 requires about 40 min for activation of MPF.
13. freeze aliquots in LN2. Store at -80 C. Will survive a few freeze/thaw cycles, but avoid excessive freeze/thawing.

Solutions

Buffer A

- 20 ml
- 50 mM Tris pH 8
- 100 mM KCl
- 5 mM MgCl2
- 5 mM DTT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