Purification of recombinant Gcn4

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Gcn4 constructs are cloned into the pET21(a) vector and have a 6-His tag expressed at the N-terminus

Gcn4 is expressed and purified from BL21(DE3)RIL cells

Day 1
Grow 10+ ml culture overnight

Day 2
1. Inoculate 1 L media with 10 ml saturated overnight
2. Grow cells to OD600 0.6 (ca. 3hrs at 37 deg)
3. Induce cells with 1 mM IPTG
4. Continue incubation for 3 hrs at 37 deg with shaking
5. Harvest cells- 5' spin at 5000 rpm in GSA
6. Wash cells with 50 ml Binding buffer
7. Resuspend cells in 20 ml Binding buffer
8. Freeze and store at -70 deg

Day 3
1. Thaw cells
2. Lyse cells with 1 pass through microfluidizer
3. Clarify extract by centrifugation- 20' spin at 10,000rpm in SS34
4. Transfer clarified extract to 50 ml tube
5. During spin prepare Ni-Sepharose: wash with 5 volumes DI water, wash with 5 volumes Binding buffer, resuspend Ni-Sepharose to 50% in Binding buffer (spins are at 500 x g for 2'-5')
6. Add 2 ml 50% Ni-Sepharose slurry to extract
7. Incubate extract with resin for 30' at RT with mixing (nutate)
8. Collect resin by centrifugation, remove supernatant and save (flow through)
9. Wash resin a total of three times using 5 volumes of Binding buffer for each wash
10. Add 2 volumes Elution buffer to resin and incubate 5' at RT with mixing (nutate)
11. Collect resin and save first elution
12. Repeat elution step 2-4 more times saving each elution

13. Analyze purification by SDS-PAGE (4-12% Bis-Tris, MES) and CBB staining- 2 microliter of each elution per lane is sufficient for gel staining along with 1microliter each of crude lysate, clarified lysate, and the flow through
14. Combine desired elutions, freeze, and store protein at -70 deg

Day 4

1. Thaw protein
2. Dilute Gcn4 to be purified 1:5 in 20 mM sodium phosphate pH 7.0 to bring NaCl concentration to 100 mM
3. Filter sample to be purified through 0.45 micron syringe filter disc
4. Prepare AKTA FPLC by established protocols (filter buffers)
5. Prepares BioRex70 column by washing first with water, then BioRex70 buffer B, and finally with 10% BioRex70 buffer B until conductivity and UV are stable
6. Prepare fraction collector as desired
7. Load Gcn4 onto FPLC
8. Run program "BioRexmGcn4NewSystem": Unicorn software, user = JamesF, password = default, program was created 8/18/05 and last modified 9/2/05

Notes

- Capacity of BioRex70 appears to be 3-4 mg of Ni-Sepharose purified Gcn4
- Gcn4 will elute from BioRex70 at approximately 0.2 mg/ml
- 1 mM PMSF and 3 mM DTT are added to all buffers for Ni-Sepharose purification on days 2 and 3
- Using protease tablets instead of PMSF does not increase Gcn4 stability during lysis and Ni-Sepharose purification
- 0.5 mM PMSF and 5 mM DTT are added to buffers for BioRex70 purification on day 4

Buffers

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

BioRex70 buffer A: 20 mM HEPES, 1 mM EDTA, 10% glycerol

BioRex70 buffer B: 20 mM HEPES, 1 mM EDTA, 10% glycerol, 1 M KCl