

CMC – Structure Initiative Protocols
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GIT1 (PBD) Expression and Purification

pGEX-GIT1c
MW = 42.8 KD w/tag
MW = 17.8 KD w/o tag
pI = 5.7
EC 280 = 0.150

Expression:

- 1) 100ml o/n culture LB/amp.
- 2) Seed 700ml TB/amp with 100ml o/n culture.
- 3) Grow until OD600=0.6-1.0.
- 4) Induce w/ 1mM IPTG.
- 5) Grow 3hr.
- 6) Harvest. 8krpm, 10min, 4°C.
- 7) Resuspend in 1xPBS. LN₂ freeze.

Purification:

- 1) Thaw 1L cells. Add PI tablet w/o EDTA. Add 2mM PMSF.
- 2) Add 0.4mg/ml lysozyme. Rock 15-30 min until thick.
- 3) Add 10mM MgCl₂. Add 0.4ug/ml DNaseI. Rock 15-30min until loose.
- 4) Add 0.1% Tx-100. Rock 15min.
- 5) Spin 16krpm, 4°C, 15min.
- 6) Wash 2 ml Glutathione Sepharose 4B beads w/PBS.
- 7) Rock supernatant with Glutathione Sepharose beads 1hr, 4 degrees.
- 8) Collect flow through.
- 9) Wash beads with 50 ml PBS.
- 10) Digest protein off beads with 50ul biotinylated thrombin/2ml PBS for 6hr at RT.
- 11) Collect eluant. Collect other fractions. Clean up thrombin with streptavidin agarose.
- 12) Run SDS PAGE.