

GIT1 (full length) Protein Purification (Baculovirus)

Baculovirus construct with 6xHis
MW 95KD

Purification:

- 1) Quick thaw 1 L cell pellet. Add 2 mM PMSF, 2 Roche PI tablets w/o EDTA. Sonicate until cells have lysed (3x20sec, on ice 20sec in between).
- 2) Add MgCl₂ to 10mM. Add 20µl Benzenase. Rock for 60min 4°C until the mixture is really runny. Save 10µl for running on gel.
- 3) Spin: 16K, 15 min, 30 ml tubes. Save supernatant and pellet. Save 10µl of each for running on a gel.
- 4) Meantime, wash Ni-NTA beads with binding buffer. Do a 1-2ml column.
- 5) Load supernatant on column. Save flow through. Save 10µl for gel.
- 6) Wash 50ml binding buffer (BB) high salt, then 50ml BB medium salt.
- 7) Wash 50ml wash buffer (20mM Imidazole).
- 8) Elute with 500mM Imidazole buffer.
- 9) Dialyze.
- 10) Run SDS PAGE. Measure protein concentration.

1X Binding Buffer (High Salt)

5mM Imidazole
500mM NaCl
10% Glycerol
3mM DTT
20mM Tris-HCl, pH 7.9

Elution Buffer (Medium Salt)

500mM Imidazole
250mM NaCl
10% Glycerol
3mM DTT
20mM Tris-HCl, pH 7.9

1X Binding Buffer (Medium Salt)

5mM Imidazole
250mM NaCl
10% Glycerol
3mM DTT
20mM Tris –HCl, pH 7.9

Dialysis Buffer (Low Salt)

20mM Tris pH7.9
250mM NaCl
10% Glycerol
5mM DTT

1X Wash Buffer (Medium Salt)

20mM Imidazole
250mM NaCl
10% Glycerol
3mM DTT
20mM Tris-HCl pH 7.9