

Production of FAK in baculovirus system

Expression:

2x500ml Sf9 cells at 2×10^6 cell/ml
10ml virus
27°C
130rpm
Grow until cells are 30% dead
Harvest: 2.5krpm, 15min, 4°C. Resuspend in 20ml PBS/200mM NaCl
LN2 freeze

Purification:

- 1) Thaw 2x500ml cell pellets. Add PI tablets w/o EDTA (crush 2 Roche tablets in 2ml PBS/200mM NaCl. Add 1ml/500ml cell pellet).
- 2) Sonicate cells 15-20sec 5-6 times on ice. Check cells under microscope to see if cells have busted open.
- 3) Spin 19krpm, 60min, 4°C.
- 4) Equilibrate 2ml column with Binding buffer 1.
- 5) Load column with supernatant.
- 6) Wash column with 50ml binding buffer 1.
- 7) Wash column with 50ml binding buffer 2.
- 8) Wash column with 30ml wash buffer.
- 9) Elute with elution buffer. Collect fractions. Pool peak. If protein starts to crash out, use 5 M NaCl to bring back into solution up to 500 mM total.
- 10) Concentrate protein to go onto S75 gel filtration column.
- 11) Gel filtration with 1xPBS/250mM NaCl/2mM β -ME. (with the S75 column you can load 500 μ l sample at a time roughly at 3-5mg/ml)

Note: if you need to concentrate the protein you will need to go up in NaCl and possible glycerol to stabilize the protein.

- 12) SDS PAGE on purification. Do protein concentration. LN2 freeze protein.

Buffers:

Binding Buffer 1

1XPBS pH7.5
200mM NaCl
2mM β -ME

Wash Buffer

1XPBS pH7.5
200mM NaCl
2mM β -ME
10% glycerol
20mM Imidazole

Elution Buffer

1XPBS pH7.5
200mM NaCl
2mM β -ME
10% glycerol
100mM Imidazole

Binding Buffer 2

1XPBS pH7.5
200mM NaCl
2mM β -ME
10% glycerol

Gel Filtration Buffer:

1XPBS pH7.5
250mM NaCl
2mM β -ME