Tat-Cre Protein Purification

Reference:

Ability of the hydrophobic FGF and basic TAT peptides to promote cellular uptake of recombinant Cre recombinase: a tool for efficient genetic engineering of mammalian genomes. Proc Natl Acad Sci U S A. 2002 Apr 2;99(7):4489-94.

- Thaw pellet (from 250ml of culture) and resuspend in 5ml lysis buffer + protease inhibitors.
- Sonicate 1 minute on, 1 minute off until lysate is no longer viscous.
- Spin the lysate down for 25 minutes at 2700 x *g*.
- Add 1 ml His-Bind Ni-charged resin (Novagen #71035) to a 15ml conical tube and spin down at 1700 x *g* for 1 minute.
- Remove supernatant and wash the resin once with 3 volumes deionized water.
- Wash the resin once with 3 volumes lysis buffer.
- Add cleared lysate to the resin (up to 5ml) and incubate for one hour at 4°C with gentle shaking.
- Spin down at 1700 x *g* for 1 minute.
- Remove supernatant and wash the resin 2X with 3 volumes of 1X binding buffer.
- Wash 2X with 3 volumes wash buffer.
- Elute bound protein 2X with 1 volume 1X elution buffer.
- Run on protein gel, transfer to PVDF, and stain with ponceau S to check purity.
- Dialyze to sterile PBS containing 10% glycerol and 50mM HEPES.
- Determine protein concentration using the Bio-Rad protein assay (catalog #500-0006) and BSA as a control.
- Store at -20°C in eppendorf sized aliquots.

*Note: there will be precipitate which we have determined to be necessary for activity but is not toxic to our cells.

Solutions

Lysis Buffer:	20mM Tris pH 7.5	0.6g
	600mM NaCl	8.75g
	20mM imidazole	0.34g
	ddH_2O	250ml

- add 25X protease inhibitor (Roche) to final concentration of 1X prior to use

Binding Buffer:	0.5M NaCl	1.5g
	20mM Tris	500ul of 2M stock
	5mM imidazole	125ul of 2M stock
	ddH_2O	to 50ml
Wash Buffer:	0.5M NaCl	1.5g
	20mM Tris	500ul of 2M stock
	60mM imidazole	1.5ml of 2M stock
	ddH_2O	to 50ml
Elution Buffer:	0.5M NaCl	1.5g
	20mM Tris	500ul of 2M stock
	0.5M imidazole	12.5ml of 2M stock
	ddH_2O	to 50ml