UL18 isolation from insect-cell media

materials
wash buffer: 150 mM NaCl, 20 mM Tris pH 7.4
pre-elution buffer: 300 mM NaCl, 50 mM Tris-Cl pH 7.4, 10 mM Imidazole, 10% glycerol
elution buffer: 300 mM NaCl, 50 mM Tris-Cl pH 7.4, 250 mM Imidazole, 10% glycerol
Ni-solution: 50 mM NiCl2, 300 mM NaCl, 50 mM Tris-Cl pH 7.4, 10% glycerol

Concentrating
- centrifuge insect media 5' 2500 rpm
- filter through a 0.45 um filter
- put media in main chamber of the concentrator (30kDa cut of)
- concentrate to 200 ml (presure ~ 15)
- add 1 l wash buffer
- concentrate to 200 ml
- add 1 wash buffer
- concentrate till 200 ml
- drain the main chamber
- add 500 ml wash buffer
- pump trough the concentrator, collecting the flowtrough (total ~ 500 ml)
- wash concentrator 3x 1 l water, 1x 500 ml 0.1 N NaOH, 3x 1 l water
- store in 500 ml 0.05% sodiumazide
- bring concentration up to 300 mM NaCl, 50 mM Tris-Cl pH 7.4, 10 mM Imidazole, 10 % glycerol

Ni-NTA column
- pass sample over Ni-NTA column (0.2-1.0 ml/min)
- wash with pre-elution buffer
- elute with elution buffer
- regenerate column with Ni-solution and wash with pre-elution buffer

FPLC
- pass eluted material over the size exclusion column
- elute with the wash buffer
- collect 4 ml fractions

BBMI column
- load sample on BBMI column 0.6 ml/min collect flow through*
- wash with wash buffer
- elute with 50 mM diethylamine pH 11
- collect piek and change lower pH with 2-3 ml 1 M Tris pH 7.4
- add 10 molar excess UL18 peptide
- run collected flow trough* over BBMI column again
- repeat washing and eluting steps do this untill no more UL18 binds
- collect piek and change lower pH with 2-3 ml 1 M Tris pH 7.4
- add 10 molar excess UL18 peptide
- change buffer to 150 mM NaCl, 20 mM Tris pH 7.4 with amicon centricons 10kDa cut of
- measure protein concentration and add 0.05% azide