Purification of His-TEV (S 219V)-Arg

Cells: BL21(DE3) RIL containing pRK793 (glycerol stock in -80°C freezer)

Buffers to prepare:

Lysis buffer: 50mM Tris, pH 7.0, 150mM NaC1, 10% glycerol

Wash buffer: lysis buffer + 10mM imidazole

Pre-elution buffer: lysis buffer + 50mM imidazole (adjust to pH 7.0)

Elution buffer: lysis buffer + 200mM imidazole (adjust to pH 7.0)

Protein expression conditions

1. **Grow cells at 37°C in LB containing 100ug/m1 ampicillin and 30ug/m1 chloramphenicol.**
2. **Induce cells when OD600 reaches 0.5-0.6 with 1mM IPTG. Lower the temperature to 30°C and grow cells for another 4 hours.**
3. **Harvest cells with PBS, TBS or lysis buffer at 4000g, 15min, 4°C and store cells in -80°C.**

Protein prep and purification

1. **Re-suspend cells in lysis buffer: 50mM Tris, pH 7.0, 500mM NaC1, 10% glycerol (20m1/L culture).**
2. **Lyse cells in a metal beaker with a sonicator on ice water. Set the amplitude at 50% with 0.5 second ON time/ 1.5 second OFF time and 5 minute total sonication time.**
3. **Centrifuge the lysed cells at 70,000g for 40 min.**
4. **Apply supernatant to pre-equilibrated Talon Co^{2+} resin column.**
5. **Wash extensively with >20CV of wash buffer. Check the flow-through with protein assay dye and wash until no color change.**
6. **Elute with pre-elution buffer (50mM imidazole) until no color change of protein assay dye.**
7. **Elute proteins with elution buffer.**
8. **Dialyze the elution against 20mM Tris, pH 7.0, 150NaCl, 10% glycerol and 5mM DTT O/N. (<5mM imidazole)**
9. **Spin down precipitated proteins, determine protein concentration, aliquot and freeze at -80°C.**

Optional: Purification with AKTA

1. **Concentrate TEV to 10-30mL, remove salt and load several times on cation ion exchange column Source 15Q. TEV is eluted between 150mM -350mM NaCl. (Buffer A: 20mM Tris, 7.0, 50mM NaC1, 1mM DTT; Buffer B: 20mM Tris, 7.0, 1M NaC1, 1mM DTT)**