Expression & Purification of His-TEV(S219V)-Arg

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BL21-RIL cells containing pRK793 are grown at 37 °C in L-broth containing 100 ug/ml ampicillin and 30 ug/ml chloramphenicol.

When the cells reach mid log phase (OD_{600} ~ 0.5), IPTG is added to a final concentration of 1 mM and the temperature is reduced to 30 °C.

After 4 hrs of induction, the cells are collected by centrifugation.

Dissolve the cell pellet in 10 ml of 50 mM PO_4 (pH 8.0) + 100 mM NaCl + 10% glycerol + 25 mM imidazole (lysis buffer) per 1 gram of wet cell paste.

Lyse the cells. We use a Gaulin cell homogenizer @ 10,000-10,500 psi for 3 passes.

Add 5% polyetheleneimine (adjusted to pH 7.9 with HCl) to a final concentration of 0.1%.

Mix by inversion and then immediately centrifuge at 15,000 x g for 30 minutes.

Apply the supernatant to a Ni-NTA column equilibrated with lysis buffer, using ca. 2 ml of resin per gram of wet cell paste.

Wash the column with 7 volumes of lysis buffer, and then elute the TEV protease with a linear gradient of lysis buffer to 50 mM PO_4 (pH 8.0) + 100 mM NaCl + 10% glycerol + 200 mM Imidizole in 10 column volumes.

Pool the appropriate fractions and then add EDTA and DTT to a final concentration of 1 mM each.

Concentrate the sample in a stirred cell using a YM10 membrane.

Load the sample onto an S-100 column equilibrated with 25 mM PO_4 (pH 8.0) + 200 mM NaCl + 10% glycerol + 2mM EDTA + 10 mM DTT (sample volume = 3% of the column volume).

Pool the appropriate fractions.

Concentrate the protease to ca. 1 mg/ml and flash freeze with liquid nitrogen.

Store at –80 °C.

The final yield should be approximately 10 mg of protein per gram of wet cell paste (ca. 30 mg/liter)