Expression & Purification of MBP-TEV(S219V)-Arg_5

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Grow BL21 or BL21(DE3) cells containing pRK1043 (MBP-TEV[S219V]-Arg5) and pRIL* in LB medium containing ampicillin (100 μg/ml) and chloramphenicol (30 μg/ml) to mid-log phase at 37 °C.

Induce production of the fusion protein by adding IPTG to a final concentration of 1 mM. At the same time, shift the temperature to 30 °C.

Harvest the cells by centrifugation after 4 hours of induction.

Resuspend 20 g of wet cell paste (from ca. 6 liters of medium) in 200 ml of 50 mM HEPES (pH 7.5), 200 mM NaCl, 1mM EDTA. Add 200 mg benzamidine and 1 "complete" protease inhibitor tablet (Roche).

Lyse the cells (we use three passes through a Gaulin cell homogenizer at 10,000 psi).

Add a solution of 5% polyetheleneimine[†] (PEI) to the lysate to give a final concentration of 0.1% PEI. Mix by inversion and then immediately pellet the precipitate by centrifugation at 15,000 x *g* for 30 min at 4 °C.

Filter the supernatant (0.45 μm).

Load the sample onto an amylose (New England Biolabs) column equilibrated with 50 mM HEPES (pH 7.5), 200 mM NaCl, 1mM EDTA. Use at least 2 ml of amylose resin per g of cell paste.

Wash the colum with equilibration buffer until the absorbance reaches baseline, and then elute the MBP fusion protein with the same buffer containing 1 M α-methylglucopyranoside (AMG)[¶].

Pool the relevant fractions and dilute 10-fold with 50 mM HEPES (pH 8.2), 5 mM DTT, 1 mM EDTA.

Load the sample onto an SP column (16 x 10 cm, 20 ml) equilibrated with the same buffer.

Apply a gradient of 0-250 mM NaCl in 50 mM HEPES (pH 8.2), 5 mM DTT, 1 mM EDTA, over 5 column volumes. The fusion protein will elute near the end of the gradient.

Pool the relevant fractions, add glycerol to a final concentration of 10% (v/v), prepare aliquots, and flash-freeze them using liquid nitrogen. Store at –80 °C.

*The tRNA accessory plasmid pRIL (from the BL21 CodonPlus strain, Stratagene) greatly improves the yield of MBP-TEV(S219V)-Arg5 fusion protein. The pRARE plasmid (from the BL21 Rosetta strain, Novagen) also works very well.

†The stock solution of PEI must be adjusted to pH 7.9 with HCl

¶We use the monosaccharide AMG to elute the fusion protein because, in contrast to maltose, it dissociates completely from MBP during the subsequent ion-exchange step. When the MBP-TEV-Arg5 fusion protein is prepared in this manner, it can be captured again on amylose resin after it is used to digest a fusion protein. The protein can be eluted with 10 mM maltose instead, but then most of it will not bind to amylose resin again.