Cabrita TEV Purification protocol

Two point mutations were introduced to produce the double mutant L56V/S135G. The mutations were introduced using the QuikChange Site Directed Mutagenesis kit (Stratagene).[1)]

- The proteins were expressed in BL21pRIPL *E. coli* (Stratagene).
- 1 L of culture.
- isopropyl β-D-thiogalactopyranoside (IPTG) (0.5 mM) induction.
- The cells were then lysed using sonication.
- The sample was then applied to 1 mL of Ni-NTA agarose (GE Healthcare) pre-equilibrated with Buffer A[2)].
- The protein was eluted with Buffer B[3)].
- The eluted protein was then applied to a Superdex 75 (16/10) column in Buffer C[4)].
- The eluted fractions containing TEV were pooled, flash-frozen in liquid nitrogen, and stored at −80°C.

1)
Cabrita et al. (2007) Protein Science 16, 2360-2367
2)
25 mM sodium phosphate (pH 8.0), 10% (v/v) glycerol, 0.2 M NaCl, and 25 mM imidazole
3)
25 mM sodium phosphate (pH 8.0), 10% (v/v) glycerol, 0.2 M NaCl, and 500 mM imidazole
4)
25 mM sodium phosphate (pH 8.0), 200 mM NaCl, 10% (v/v) glycerol, 5 mM β-mercaptoethanol

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