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, 200 mM NaCl, and 25 mM imidazole

3)

25 mM sodium phosphate (pH 8.0), 10% (v/v) glycerol, 200 mM NaCl, and 500 mM imidazole

4)

25 mM sodium phosphate (pH 8.0), 10% (v/v) glycerol, 200 mM NaCl, 5 mM β-mercaptoethanol