

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 A²⁾.
- The protein was eluted with Buffer B³⁾.
- The eluted protein was then applied to a Superdex 75 (16/10) column in Buffer C⁴⁾.
- 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 Na₂PO₄ (pH 8.0), 200 mM NaCl, 10% (v/v) glycerol, 5 mM β -mercaptoethanol

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