TEV Protease

Histidine-tagged recombinant, expressed in E. coli, rigorously purified via three rounds of chromatography.

Information (from SIGMA-ALDRICH manual):

TEV protease, originally isolated from Tobacco Etch Virus, is a member of the family of C4 peptidases. Three proteolytic enzymes are released from the N-terminus of the TEV polyprotein: P1, HC-Pro, and Nuclear Inclusion α (NIα).
In its native form, TEV protease is a 27 kDa catalytic domain that was originally noted as the C-terminal portion of the NIα part of the TEV polyprotein.[1,2]

TEV protease specifically cleaves proteins within a seven-residue optimal recognition sequence. This sequence is:

Glu-Asn-Leu-Tyr-Phe-Glu-Gly-Ser (ENLYFQ↓G/S)

The 7th residue can be either Gly (G) or Ser (S), and proteolytic cleavage occurs between the Glu and Gly/Ser residues.[1] Because of this strict sequence recognition specificity, TEV protease has thus become a useful tool to cleave recombinant proteins that are expressed as fusion proteins with this sequence between the carrier domain and the protein of interest.[2]

TEV protease has activity in the pH range of 6-9. At pH <5, TEV protease is inactive.[1] Under *in vitro* conditions, native TEV protease has optimal activity in the absence of monovalent salts (e.g., NaCl).[3] However, TEV protease may be successfully used in the presence of NaCl at concentrations up to 200 mM.[1,3]

TEV Protease, in its native form, has limited solubility in aqeous media. Many studies have indicated that the use of different tags, to express TEV protease in tagged recombinant form, greatly facilitate the solubility of TEV protease in aqeous media.[2,4]

Cleavage Protocol (Wedekind Lab):

1. We keep protein stocks in concentration of ~2 mg/ml stored at -80 °C freezer in 200 µl aliquots in frozen$_{(S)}$ form. To thaw the aliquot, submerge the Eppendorf tube in a floaty in room temperature water and incubate for ~1 min.
2. This is the rule of units of activity we've been following: 1 unit of TEV protease to 100 units of protein to cleave (1:100 w/w).
3. Since the enzyme likes to work better under low salt conditions, it is recommended that the concentration of monovalent salts does not exceed 200 mM in cleavage reaction.
4. In the Wedekind lab, we've had great success (cleavage approaching 100%) cleaving ~40 mg of protein (at ~1 mg/ml) with 350 µg of TEV protease (200 µl

aliquot) by mixing TEV protease into protein solution (pipet well to mix thoroughly) and leaving the reaction at 4 °C undisturbed (still), WITHOUT rocking, for ~16 hours or overnight. Of course, it would be recommended to perform a test of different time-points, as it may depend from case to case, depending on many things, including TEV site accessibility.

1. Waugh, D.S., *Protein Expr. Purif.,* **80(2)**, 283-293 (2011).
2. Cesaratto, F. *et al*., K. *Biotechnol*., **231**, 239-249 (2016).
3. Nallamsetty, S. *et al., Protein Expr. Purif*., **38(1)**, 108-115 (2004).
4. Zdanov, A.S. *et al*., *Russ. J. Bioorg. Chem*., **29(5)**, 415-418 (2003).