TEV Protease FAQ

What protease inhibitors are known not to affect TEV?

PMSF and AEBSF (1mM), TLCK (1mM), Bestatin (1mg/ml), pepstatin A (1mM), EDTA (1mM), and E-64 (3mg/ml), "complete" protease inhibitor cocktail (Roche). Zinc will inhibit the activity of the enzyme at concentrations of 5 mM or greater. Reagents that react with cysteine (e.g., iodoacetamide) are potent inhibitors of TEV protease.

What are typical reaction conditions?

The "standard" reaction buffer for TEV protease is 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA and 1mM DTT. The duration of the cleavage reaction is typically overnight, although lots of cleavage will happen in the first few hours and prolonged incubation times may not lead to proportional increases in cleavage. TEV protease is maximally active at 34 °C, but we recommend performing the digest at room temperature (20 °C) or 4 °C. TEV protease is only three-fold less active at 4 °C than at 20 °C [Nallamsetty et al., 2004].

How much TEV protease should I use?

A good rule of thumb is 1 OD280 of TEV protease per 100 OD280 of substrate for an overnight digest. Perform a small-scale reaction first, if possible, to gauge the efficiency of processing. In especially difficult cases, we have used as much as 1 OD280 of TEV protease per 5 OD280 of substrate. This is feasible because TEV protease is not a promiscuous enzyme.

Can I do an on-column cleavage?

On-column cleavage is possible but comparatively inefficient. TEV protease is not especially stable and has a tendency to bind nonspecifically to column materials. The addition of "stabilizers" like sorbitol, glycerol, or salt may be helpful. For an example of on-column cleavage by immobilized TEV protease, see Puhl et al. (2009).

How sensitive is TEV protease to other additives and reaction parameters?

TEV protease has a relatively flat activity profile at pH values between 4 and 9. It is 50% as active in 0.5 M NaCl as it is in the absence of salt [Nallamsetty et al., 2004]. TEV protease is maximally active at 34 °C, but we recommend performing digests at room temperature (20 °C) or 4 °C. The activity of TEV protease is approximately 3-fold greater at 20 °C than at 4 °C [Nallamsetty et al., 2004]. It will

tolerate a range of buffers, including phosphate, MES, and acetate. TEV protease is not adversely affected by the addition of glycerol or sorbitol (up to at least 40% w/v). TEV protease is sensitive to some detergents [Mohanty et al., 2003; Lundback et al., 2008].

What about disulfide bonds?

If the target protein is expected to contain disulfide bonds, DTT should not be used in the reaction. Under these conditions, DTT can be replaced with a redox buffer like 3 mM glutathione/0.3 mM oxidized glutathione, which should maintain the disulfide bonds while providing enough reducing power for TEV protease to work.

What about zinc fingers?

Both EDTA and DTT are strong metal chelators, and consequently they might be expected to strip the zinc ions from weaker zinc finger motifs. We recommend that DTT be replaced by a monothiol such as glutathione or beta-mercaptoethanol when working with zinc fingers. EDTA should be replaced by a weaker metal chelator such as citrate. Ming Zhou from the Morrison laboratory at NCI-Frederick found that the following buffer supports TEV protease activity and is expected to be very kind to zinc fingers (20 mM Tris-HCl, pH 7.4, 10 µM $ZnCl_2$, 200 mM NaCl, 5 mM citrate, 5 mM 2-mercaptoethanol).

How can I remove TEV protease after digesting a fusion protein substrate?

When working with an affinity-tagged form of TEV protease, an obvious option is to absorb it to the appropriate affinity resin. Don't forget that DTT and EDTA are incompatible with IMAC and therefore must be removed first. Because of the C-terminal polyarginine tag, both forms of the S219V mutant TEV protease that we use will bind very tightly to a cation exchange resin, even at pH 8-9. Under these conditions, the vast majority of proteins will fail to adhere to the resin. Gel filtration may also work, depending on the size of the target protein and the form of TEV protease used.

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