

TEV protease production and cleavage

The 27 kDa catalytic domain of the viral NIa polypeptide of Tobacco Etch Virus (TEV) is a cysteine protease, with a Cys-Asp-His catalytic triad, that cleaves ENLYFQ/G (or ENLYFQ/S) between the P1 Gln and the P1' Gly/Ser. The autolysis-resistant S219V variant (Kapust et al., 2001) is about 100-fold more stable than the wild-type protease, and twice as active. Its narrow specificity, broad activity window (pH 4.0 to pH 9.0, 4–34 °C), and tolerance of common purification buffers make TEV the standard choice for removing affinity tags downstream of His-, MBP-, or GST-capture.

The construct is suitable for cleavage in solution. For on-column cleavage, PreScission (3C, LEVLFQ/GP) or SUMO (Ulp1) ⓘ SOP0048 are preferred.

A single 1 L autoinduction prep yields tens of milligrams of protease, enough to cleave hundreds of milligrams of tagged target, at a fraction of the cost of commercial protease.

Risk assessment

- ▷ Wear cryo-gloves for liquid-nitrogen handling
- ▷ Wear gloves, safety glasses, lab coat



Reviewed: Oct 12, 2026

Procedures

>> Preparative expression

- | | |
|---|--|
| ☐ Erlenmeyer flask, 1–2 L, baffled | ☐ <i>E. coli</i> BL21(DE3) |
| – or: Fermenter, 4 L capacity | ☐ pOPIN-His6-MBP-(3C)-TEV, 70.3 kDa |
| ☐ Centrifuge bottle, 1 L | – or: pRK793, MBP-(TEV)-His6-TEV-Arg5, 28 kDa, Addgene |
| ☐ TB autoinduction medium ⓘ R0205 | – or: pRK1043, MBP-TEV-Arg5, 70.0 kDa, Addgene |

- (1.) Inoculate 50 mL LB containing ampicillin at 100 mg/L with a single fresh colony of *E. coli* BL21(DE3) carrying the TEV expression plasmid. Incubate overnight at 37 °C, 180 rpm. ☾

Hint: All three plasmids listed carry ampicillin resistance. Transform fresh for each preparation ⓘ SOP0034 rather than working from a glycerol stock. Colonies older than two or three days accumulate non-expressing variants.

- (2.) Inoculate 1 L TB autoinduction medium with 10 mL of the overnight pre-culture in a baffled Erlenmeyer flask. Incubate at 20 °C, 180 rpm, for 18–24 h, typically overnight. ☾

- (3.) Harvest the cells at 6000 × g for 15 min at 4 °C. Discard the supernatant and weigh the wet pellet.

Hint: Pellets may be stored at –80 °C for months before lysis.

>> Lysis

- | | |
|--|---|
| ☐ TEV lysis buffer, pH 8.0 ⓘ R0206 | ☐ DNase I, 10 U/μL stock |
| ☐ 100 mM 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride ⓘ R0213 | ☐ 1 M Magnesium chloride ⓘ R0031 |

- (1.) Resuspend the pellet in 5 mL cold TEV lysis buffer per 1 g wet mass. Supplement AEBSF to 0.5 mM, MgCl₂ to 2 mM, and DNase I to 40 U/mL final. Keep the lysate on ice.

This is why: DNase I is Mg²⁺-dependent, so TEV lysis buffer must be spiked. The magnesium is removed on the column and does not reach the protease product.

- (2.) Lyse the cells by sonication on ice (0.125 in horn; 6 cycles of 30 s on, 60 s off, at 40% amplitude) or by two passes through a high-pressure homogenizer.

Hint: Final lysate volume from 1 L TB-AI is typically 50–75 mL.

(3.) Clear the lysate by centrifugation at $40\,000 \times g$ for 45 min at 4 °C.

>> Affinity chromatography (HisTrap FF on ÄKTA)

☐ FPLC system	☐ TEV elution buffer, pH 8.0  R0207
☐ HisTrap FF (crude) column, 5 mL capacity	

- Capture the fusion on Ni^{2+} and elute it with a shallow imidazole gradient, using the HEPES-based buffers below in place of the phosphate-based ones in  SOP 0045.

(1.) Filter the cleared lysate through a 0.45 μm filter immediately before loading.

Critical: An unfiltered lysate clogs the HisTrap FF crude inlet within minutes at the loading flow rate; do not skip filtration. 

(2.) Run the FPLC with TEV lysis buffer as the binding buffer and TEV elution buffer as eluent. Alternatively, use gravity-flow Ni-NTA as a fallback. The settings below are for the 5 mL column.

- Equilibrate with 5 vol lysis buffer at 1 vol/min.
- Load the cleared, filtered lysate, 50–75 mL, at 0.4 vol/min.

Hint: No separate imidazole spike is needed before loading: the lysis buffer already carries 20 mM imidazole.

- Wash with at least 10 vol lysis buffer at 1 vol/min, until A280 returns to baseline.
- Elute at 1 vol/min with a four-segment gradient, collecting 0.4 vol fractions: 0–10% eluent over 2 vol, 10–50% over 15 vol, 50–100% over 1 vol, then 100% for 5 vol.

Hint: The His6-MBP-TEV peak elutes in the shallow 10–50% segment, at 150–250 mM imidazole.

(3.) Analyze peak fractions by SDS-PAGE  SOP 0007. Pool fractions containing a single sharp band at 70 kDa (His6-MBP-TEV) or 28 kDa (His6-TEV-Arg5).

Critical: The 3C linker in pOPIN is not a TEV substrate, so the fusion protein stays intact. The pRK793 self-cleavage construct frees His-MBP at 44 kDa; this is a contaminant and must not be pooled. 

>> Dialysis and concentration

☐ TEV storage buffer, pH 8.0  R0208	☐ Glycerol, anhydrous  R0022
☐ Dialysis membrane tube	☐ 500 mM Tris(2-carboxyethyl)phosphine hydrochloride  R0215

(1.) Pool the pure fractions. Expected pooled concentration is 2–4 mg/mL by A280.

(2.) Add TCEP to 0.5 mM. Transfer the material to a dialysis tubing.

(3.) Dialyze overnight at 4 °C against TEV storage buffer at a sample-to-buffer ratio of 1 : 25 or higher. 

Critical: 1.5 L dialysis buffer brings a sample of up to 60 mL below 20 mM imidazole. Split larger pools across two baths. 

(4.) Recover the dialysate. The in-bag TCEP has dialysed out by now, so add TCEP again to 0.5 mM final. Clear the dialysate at $20\,000 \times g$ for 15 min at 4 °C to remove any precipitate.

(5.) Determine the concentration spectrophotometrically at 280 nm. Bring to 2 mg/mL with TEV storage buffer. If below 2 mg/mL, concentrate over an ultrafiltration device ( SOP 0045).

Critical: Before concentrating, raise sodium chloride to 1 M with the 5 M stock; the protease otherwise precipitates. Dilute back to 500 mM with TEV storage buffer once the target concentration is reached, so the lot is stored at the stated composition. 

Hint: The molar extinction coefficient of His6-MBP-TEV is $98\,570\text{ M}^{-1}\text{ cm}^{-1}$ and has an absorbance of 1.39. His6-TEV-Arg5 has a molar extinction coefficient of $31\,970\text{ M}^{-1}\text{ cm}^{-1}$ (absorbance 1.12).

Quality assurance: A typical His-TEV preparation yields on the order of 30 mg per 1 L of culture. 

- (6.) Bring the lot to 50% glycerol (v/v) by adding 0.9 mL anhydrous glycerol per 1 mL of lot, the storage buffer already contributes 10%. Mix thoroughly; the protease is then at about 1 g/L.

This is why: Commercial TEV preparations are supplied at 50% glycerol. At that concentration the lot does not freeze solid, so thawing costs no activity and a tube tolerates being sampled more than once.

>> Aliquot, storage, and lot record

- (1.) Aliquot the protease into 50–100 μL portions and store at $-20\text{ }^{\circ}\text{C}$. Alternatively, snap-freeze each tube in liquid nitrogen and store at $-80\text{ }^{\circ}\text{C}$.
- (2.) Record the following lot metadata in the protease lot log:
 - Lot number as MM/YYYY, following the labelling convention in [SOP0030](#)
 - Date of preparation and contributor
 - Final concentration (mg/mL) and total yield (mg)
 - Number and size of aliquots
 - Specific activity (mg protease required to cleave 1 mg of 70 kDa substrate at $4\text{ }^{\circ}\text{C}$ overnight)
 - Storage buffer composition and storage location

>> Standard activity assay

☐ TEV cleavage buffer, pH 8.0 R0209	☐ TEV-cleavable test substrate
--	---

- (1.) Dilute the TEV-cleavable test substrate to 0.5 mg/mL. Any purified His-MBP fusion carrying an EN-LYFQ/G site will work. Activity is normalized to a nominal substrate mass of 70 kDa.

Critical: One lot test consumes about 240 μL of the diluted substrate. The substrate must be purified before the test is started. 

- (2.) Thaw two separate TEV protease aliquots of the lot. From each, prepare 50 μL at 0.4 mg/mL in cleavage buffer. For a lot at 1 g/L this is 20 μL lot plus 30 μL buffer.

Critical: The two aliquots carry the replication of the assay. Splitting one aliquot into two series replicates the pipetting but not the aliquot, hence reporting a precision the lot does not have. 

- (3.) Carry each replicate down a five-point two-fold series in reaction tubes [SOP0031](#). Pre-fill tubes with 25 μL cleavage buffer, transfer 25 μL from tube 1 into tube 2, mix, and continue with a fresh tip:

Tube	TEV concentration	TEV mass per assay	TEV per 1 mg substrate
1	0.400 mg/mL	2.000 μg	0.200 mg
2	0.200 mg/mL	1.000 μg	0.100 mg
3	0.100 mg/mL	0.500 μg	0.050 mg
4	0.050 mg/mL	0.250 μg	0.025 mg
5	0.025 mg/mL	0.125 μg	0.013 mg

- (4.) Transfer 5 μL of each dilution of both series into fresh tubes. Add 20 μL of the 0.5 mg/mL substrate dilution (10 μg substrate; 25 μL total). Mix gently, spin down briefly, and incubate overnight at $4\text{ }^{\circ}\text{C}$.
- (5.) After 18 h, stop each reaction with 8 μL $4\times$ SDS-PAGE sample buffer [SOP0007](#) and heat to $95\text{ }^{\circ}\text{C}$ for 5 min. Prepare a substrate only (20 μL substrate dilution plus 5 μL cleavage buffer) and protease only (5 μL of tube 1 of either series plus 20 μL cleavage buffer) control. 

Critical: The protease and the uncleaved substrate differ by under 2 kDa and co-migrate on this gel. Use the protease-only lane to place that band, so the protease is not read as residual substrate. 

- (6.) Resolve both series and the two controls on one 12% SDS-PAGE gel [SOP0007], loading 10 µL per lane. Stain with Coomassie [SOP0010]. Look for the substrate band at 68.8 kDa to disappear in favor of the His-MBP (44 kDa) and Rab7 (23.7 kDa) product bands.

Quality assurance: The twelve samples fit a 12-well comb; cast a 15-well gel to carry a molecular-weight marker alongside them. Keeping both series on one gel means the two endpoints are compared under identical staining.

Optional: Using TEV protease for tag cleavage

- | | |
|--|--|
| ☐ Dialysis tubing or cassette | ☐ EDTA, 0.5 M stock, pH 8.0 |
| ☐ Reverse IMAC column | |

- (1.) Verify that the fusion places ENLYFQ/G between the tag and the target, with at least three residues of flexible linker between the cleavage site and the folded domain of the target. Cleavage leaves the Gly on the target N-terminus.

- (2.) Match the cleavage mixture to the following reaction conditions:

- Buffering system: Tris, HEPES, phosphate, MES, or acetate buffer (pH 4.0 to pH 9.0)
- Temperature: 4–34 °C; default 4 °C overnight
- Reductant: 1 mM TCEP, or 1–5 mM DTT
- Tolerated protease inhibitors: PMSF and AEBSF (1 mM), TLCK (1 mM), pepstatin A (1 mM), bestatin (1 mg/mL), E-64 (3 mg/mL), Roche "complete" cocktail (1 ×)

Hint: Two target classes override the reductant choice: for disulfide-bonded targets replace DTT with a redox buffer (3 mM reduced and 0.3 mM oxidized glutathione), for zinc-fingers replace DTT with β-mercaptoethanol and EDTA with citrate.

- (3.) Conditions incompatible with TEV protease activity:

- Monovalent salt exceeding 300 mM NaCl
- Any amount of Mg²⁺ or Mn²⁺ must be quenched with EDTA; keep Zn²⁺ below 5 mM
- Imidazole: exceeding 150 mM
- Glycerol or sorbitol: exceeding 40% (w/v)
- Iodoacetamide and NEM are potent TEV protease inhibitors
- For detergents, verify per detergent before scaling up, some are inhibitory

- (4.) Add TEV to the pooled chromatographic fractions containing the tagged target protein at the ratio indicated by the current lot's activity assay (typically 0.05 mg TEV per 1 mg target). Take a zero-time sample for SDS-PAGE, mix with sample buffer, and freeze immediately.

This is why: On-column cleavage is inefficient here: TEV binds nonspecifically to column materials; the His6-MBP-TEV also competes with the tagged substrate for the same Ni²⁺ sites, and the EDTA that protects TEV from divalent metals strips the resin. PreScission (3C) and SUMO (Ulp1) are the usual choices for on-column work.

- (5.) Transfer the cleavage mixture to pre-rinsed dialysis tubing or a dialysis cassette. Add TCEP to 0.5 mM directly into the dialysis vessel before sealing. Dialyze against the target's storage or next-step buffer at a sample-to-buffer ratio of at least 1 : 100 for 16 h at 4 °C.

Hint: Cleavage during dialysis removes imidazole and any low-molecular-weight inhibitors as the reaction runs.

- (6.) Remove the His6-MBP-TEV protease and any released His-tagged portion of the substrate by reverse IMAC [SOP0045]. The cleaved, tag-free target appears in the flow-through.

Critical: Remove EDTA and strong reductants such as DTT before reverse IMAC; EDTA strips Ni²⁺ from the resin and DTT reduces it. The preceding dialysis does this provided the dialysis buffer contains neither. TCEP at 1 mM, as used throughout this protocol, is tolerated on immobilized nickel and does not have to be removed.

- (7.) Confirm complete cleavage by SDS-PAGE ☞ SOP0007 against the zero-time sample. Expect loss of the tagged precursor band and gain of two product bands. ✕

Analyses

- Record the lot's specific activity as the highest endpoint with no detectable 68.8 kDa band.

Critical: The two endpoints must agree within one tube. On a discrepancy of two tubes or more, run a third series from a third aliquot and report the median; do not average the three, because a two-fold ladder is an ordinal scale and the mean of two rungs is not a measured value. ←

- Reject the lot and repeat the preparation if either series leaves uncleaved substrate at the highest TEV concentration. Otherwise release the lot and use its recorded specific activity to dose every subsequent cleavage reaction.

Hint: A typical preparation will fully cleave at 0.05 mg TEV per 1 mg substrate. A lot that needs a higher tube is still usable; it simply consumes proportionally more protease per digest.

Materials

Preparative expression

Cultivation vessel

- Erlenmeyer flask, 1–2 L, baffled
- or Fermenter, 4 L capacity

Centrifuge bottle, 1 L

TB autoinduction medium ☞ R0205, Store at 4 °C.

E. coli BL21(DE3)

TEV (S219V) expression plasmid

- pOPIN-His6-MBP-(3C)-TEV, 70.3 kDa, His6-MBP joined by a 3C linker (LEVLFQ/GP), which TEV cannot cleave; $\epsilon_{280} = 98\,570\text{ M}^{-1}\text{ cm}^{-1}$
- or pRK793, Addgene, 8827, MBP-(TEV)-His6-TEV-Arg5, 28 kDa, RRID Addgene_8827
- or pRK1043, Addgene, 8835, MBP-TEV-Arg5, 70.0 kDa, RRID Addgene_8835

Lysis

TEV lysis buffer ☞ R0206, pH 8.0, Store at 4 °C.

4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride ☞ R0213, 100 mM

DNase I, 10 U/μL stock

Magnesium chloride ☞ R0031, 1 M

Affinity chromatography (HisTrap FF on ÄKTA)

FPLC system

- e.g. ÄKTA pure, Cytiva

HisTrap FF (crude) column, 5 mL capacity

- HisTrap FF crude, Cytiva, 17-5286-01

TEV elution buffer ☞ R0207, pH 8.0, Store at 4 °C.

Dialysis and concentration

TEV storage buffer ☞ R0208, pH 8.0, Store at 4 °C.

Dialysis membrane tube, MWCO 12–14 kDa

- e.g. Spectra/Por® 4, Spectrum Labs, Spectra/Por® dialysis tubing

Glycerol ☞ R0022, anhydrous

Tris(2-carboxyethyl)phosphine hydrochloride ☞ R0215, 500 mM

TEV protease production and cleavage

Standard activity assay

TEV cleavage buffer  R0209, pH 8.0, Store at 4 °C.

TEV-cleavable test substrate, The reference substrate is a His-MBP-(TEV)-Rab7 fusion, 68.8 kDa, with a molar extinction coefficient at 280 nm of $95\,020\text{ M}^{-1}\text{ cm}^{-1}$; on cleavage it releases free Rab7 (23.7 kDa) and free His-MBP (44 kDa), both readily resolved on a 12% SDS-PAGE gel.

Using TEV protease for tag cleavage

Dialysis tubing or cassette, MWCO below the mass of the freed target and well below the protease mass; 12–14 kDa as used here; pre-rinsed

- Dialysis membrane tube
- Dialysis cassette

Reverse IMAC column, for reverse-IMAC removal of cleaved tag and TEV

- HisTrap or Ni-NTA column

EDTA, 0.5 M stock, pH 8.0

Recipes

TB autoinduction medium (TB-AI)

Amount	Ingredient		Stock	Final
2 mL	MgSO ₄	 [10034-99-8]	1 M	2 mM
1 mL	Ampicillin	  R0004	100 g/L	100 mg/L
	Skin sensitization (H317)			
500 µL	Lactose	 [63-42-3]	20%	0.01%
To 1 L Terrific broth (TB) medium, autoclaved				

Store at 4 °C.

TB autoinduction medium (TB-AI)

 2 mM MgSO₄,  100 mg/L Ampicillin,
 0.01% Lactose



WARNING



Skin sensitization

 Hold for EHS review before disposal

Date: Sign: R0205

TEV lysis buffer, pH 8.0

Amount	Ingredient		Stock	Final
25 mL	HEPES	⚠ R0024	1 M	50 mM
30 mL	Sodium chloride (NaCl)	⚠ R0046	5 M	300 mM
1.67 mL	Imidazole	[288-32-4] ⚠ R0027	6 M	20 mM
	Skin corrosion (H314); Reproductive toxicity (H360)			
1 mL	TCEP	☐ ⚠ R0215	500 mM	1 mM
	Skin corrosion (H314)			
To 500 mL	Water, reagent-grade			

Adjust to pH 8.0 with NaOH after imidazole addition. Chill to 4 °C but do not place on ice; the buffer precipitates. Add TCEP immediately before use. Store at 4 °C.

TEV lysis buffer

50 mM HEPES, 300 mM NaCl, 20 mM Imidazole,
 1 mM TCEP, pH 8.0



DANGER



Reproductive toxicity

 Collect as HAZARDOUS WASTE
 DO NOT wash into sewer

Date: Sign: R0206

TEV elution buffer, pH 8.0

Amount	Ingredient		Stock	Final
10 mL	HEPES	◇ R0024	1 M	50 mM
12 mL	Sodium chloride (NaCl)	◇ R0046	5 M	300 mM
16.7 mL	Imidazole	[288-32-4] ◇ R0027	6 M	500 mM
	Skin corrosion (H314); Reproductive toxicity (H360)			
0.4 mL	TCEP	□ ◇ R0215	500 mM	1 mM
	Skin corrosion (H314)			
To 200 mL	Water, reagent-grade			

Adjust to pH 8.0 with HCl after imidazole addition; chill to 4 °C. Add TCEP immediately before use. Store at 4 °C.

TEV elution buffer

50 mM HEPES, 300 mM NaCl, 500 mM Imidazole, □ 1 mM TCEP, pH 8.0



DANGER



Reproductive toxicity

- Collect as HAZARDOUS WASTE
- DO NOT wash into sewer

Date: Sign: R0207

TEV storage buffer, pH 8.0

Amount	Ingredient		Stock	Final
75 mL	HEPES	◇ R0024	1 M	50 mM
150 mL	Sodium chloride (NaCl)	◇ R0046	5 M	500 mM
300 mL	Glycerol	◇ R0022	50%	10%
To 1.5 L	Water, reagent-grade			

Adjust to pH 8.0; chill to 4 °C before dialysis. TCEP is added directly to the dialysis bag, not to the bulk buffer. Store at 4 °C.

TEV storage buffer

50 mM HEPES, 500 mM NaCl, 10% Glycerol, pH 8.0



No GHS hazards at the indicated concentrations

- Dilute below the discharge limit
- Flush to drain with excess water

Date: Sign: R0208

TEV cleavage buffer, pH 8.0

Amount	Ingredient		Stock	Final
5 mL	HEPES	◇ R0024	1 M	50 mM
10 mL	Sodium chloride (NaCl)	◇ R0046	5 M	500 mM
333 µL	Imidazole	[288-32-4] ◇ R0027	6 M	20 mM
	Skin corrosion (H314); Reproductive toxicity (H360)			
10 mL	Glycerol	◇ R0022	50%	5%
200 µL	TCEP	□ ◇ R0215	500 mM	1 mM
	Skin corrosion (H314)			
To 100 mL	Water, reagent-grade			

Store at 4 °C. **Note:** For TEV standard activity test only.

TEV cleavage buffer

50 mM HEPES, 500 mM NaCl, 20 mM Imidazole, 5% Glycerol, □ 1 mM TCEP, pH 8.0



DANGER



Reproductive toxicity

- Collect as HAZARDOUS WASTE
- DO NOT wash into sewer

Date: Sign: R0209

Troubleshooting

Using TEV protease for tag cleavage

In Step 1:

- Inefficient cleavage
 - Match the TEV cleavage site exactly: even conservative substitutions at P2 (Phe to Tyr) or P4 (Leu to Phe) reduce processing by about two orders of magnitude.

In Step 7:

- Cleavage stalls at 50% overnight
 - Confirm sample is within the TEV buffer windows above; in particular, check for residual imidazole exceeding 150 mM and undeclared divalent metals.
 - Use an unopened aliquot; a tube stored at -80°C and thawed once already has lost activity.
 - Increase the TEV-to-target ratio twofold and extend the incubation to 24–36 h at 4°C , or warm to 16°C if the target tolerates it.
- Target precipitates during cleavage
 - Tagged partners such as MBP, SUMO, and GST are solubilizing, so the free target may be less soluble. Keep the target concentration below 1 mg/mL, raise salt to 300–500 mM NaCl, add 10% glycerol before TEV addition, or drop the cleavage temperature to 4°C if it was higher.

List of references

R.B. Kapust, J. Tözsér, J.D. Fox, D.E. Anderson, S. Cherry, T.D. Copeland, and D.S. Waugh, *Protein Eng.* **14**(12), 993–1000 (2001).

Change log

2026-05-24	Benjamin C. Buchmuller	Initial version. Consolidates the Daniel Summerer lab "Production and Activity Test of TEV Protease" (v15) preparative workflow and use notes into a single protocol. Cleavage chemistry and the buffer-compatibility windows are supplemented from the David Waugh lab "TEV Protease FAQ" (2010).
2026-10-12	Benjamin C. Buchmuller	Activity assay recast as a five-point two-fold dilution series run in duplicate from separate aliquots, which centres the reference lot in the series and makes the lot-release criterion explicit. Imidazole ingredients tagged by CAS, so the buffer cards classify its reproductive toxicity instead of reporting an unspecified concentration.

From the *Lab Protocols* collection by B. C. Buchmuller and contributors (2026). Licensed under Creative Commons Attribution Share Alike 4.0 International; see <https://creativecommons.org/licenses/by-sa/4.0/> for the terms.

For research use. Provided in good faith, without warranty or liability for any use or results. Users are responsible for compliance with local regulations and institutional policies. Where a brand name, manufacturer, or specific source is cited, it is given as an example and does not imply that other suitable equivalents are excluded.

Current when printed. Visit <https://benjbuch.github.io/check/> or scan the QR code to check for updates.



eeab5ab