Cold-Active TEV Protease: Engineered for higher performance at low temperatures

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Introduction

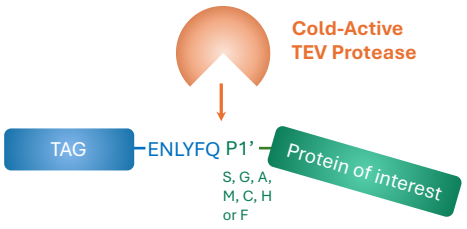
Cold-Active TEV Protease (NEB #P8118S) has been engineered specifically for higher activity at lower temperatures, offering up to 3X faster cleavage of fusion protein substrates than standard TEV proteases at 4°C. We investigated:

1- Its performance on different targets showing 3 to 4x improved specific activity at 4°C than standard TEV Protease

2- Its ability to cleave an affinity tag from a protein of interest immobilized on an affinity resin, enabling gentle on-resin elution of a tag-free target protein

3- Its efficiency of removal from a reaction with Ni-NTA beads using its 7X His-tag

Cold-Active TEV Protease is ideal for rapidly cleaving affinity tags from recombinant proteins or peptides, especially when they are thermosensitive, and can be used in wide range of temperatures (4 to 37°C) and buffers.

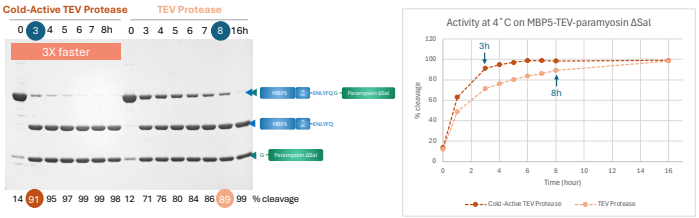


NEB #P8118S

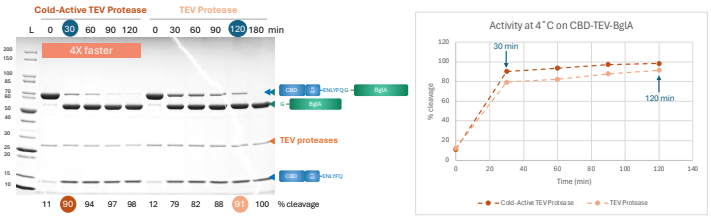
- 10,000 U/mL (1 mg/mL)
- 1000 Units (100 µL)
- Reaction Buffer 10x (1 mL)

3-4X faster cleavage at 4°C

1 **MBP5-TEV-Paramyosin ΔSal is cleaved to 90% in 3 hrs with Cold-Active TEV Protease.**



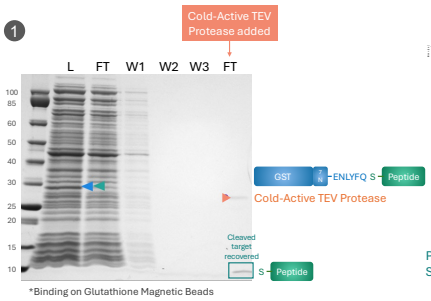
2 **CBD-TEV-BglA is cleaved to 90% in 30 min with Cold-Active TEV Protease.**

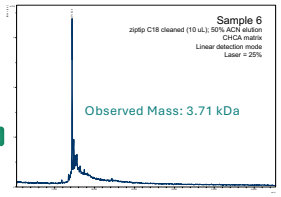


*Cold-Active TEV Protease and TEV Protease (NEB #P8112) were incubated with both substrates at 4°C in 1X TEV Protease reaction buffer at a **ratio 1:16 (1 µg or 10 U of enzyme for 16 µg of substrate)**. Samples were collected at various times, and enzymatic reactions were stopped with Blue Protein Loading Dye (NEB #B7703). Cleavage efficiency was analyzed by SDS-PAGE in which each lane was loaded with 2 µg of substrate and 1.25 units (0.125 µL) of enzyme. The percentage of substrate cleavage was determined by densitometry.*

Cleaves immobilized targets from affinity resins

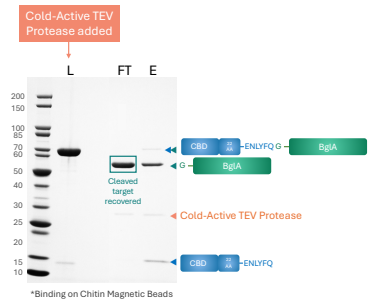
1





Peptide 33 aa (MW 3.71 kDa):
SKEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK

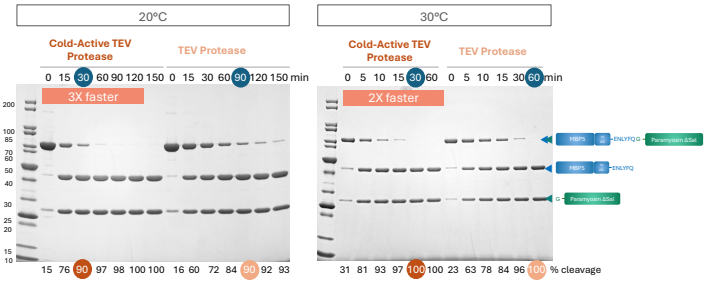
2



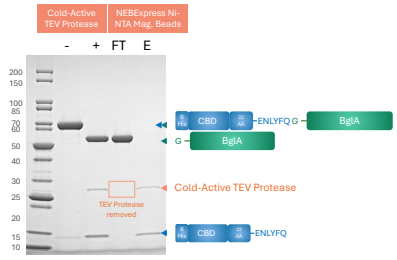
1 *GST-TEV-Peptide was expressed in vitro using NEBExpress Cell-Free E. coli Protein Synthesis System (NEB #E5360) and purified on Glutathione Magnetic Agarose Beads (Thermo Scientific) at 37°C for 4h. 20 µL Glutathione Magnetic beads were used to capture the GST-TEV-Peptide target. After few washes, 10 U (1 µL) of Cold-Active TEV Protease were added for 1h at 25°C (50 µL reaction on a rotator) allowing the peptide to be collected in the flow through (FT), along with the protease. Peptide MW was confirmed by MALDI-TOF mass spectrometry.*

2 *Purified CBD-TEV-BglA was immobilized on Chitin Magnetic Beads (NEB #E8036) followed by the addition of Cold-Active TEV Protease at a ratio 1:15 (2 µg or 20 U for 30 µg of substrate). After 1h at 25°C on a rotator, the flow through (FT) was collected containing the substrate BglA and the protease. The Chitin Magnetic Beads were then stripped in presence of Blue Protein Loading Dye (NEB #B7703S), containing the CBD-tag and some leftovers of BglA and protease (E)*

Efficient in a wide range of temperatures



Efficient removal of Cold-Active TEV Protease with IMAC



Cold-Active TEV Protease and the substrate HisCBD-TEV-BglA were incubated at a ratio 1:15 (1 µg or 10 U of enzyme with 15 µg of substrate) for 2h at 4°C in 1X TEV Protease reaction buffer. After cleavage of the substrate, the reaction was mixed with Ni-NTA Magnetic Beads (NEB #S1423) for 30 min at 4°C. As Cold-Active TEV Protease carries a 7X His tag, the enzyme was removed from the reaction, along with the His-CBD tag (flow through – FT). The Ni-NTA magnetic beads were then stripped in presence of Blue Protein Loading Dye (NEB #B7703S), revealing the captured His-tagged Cold-TEV Protease and the cleaved His-CBD (E).

Takeaways

- **Cold-Active TEV Protease is:**
  - **Faster than TEV Protease, especially at 4°C – Save a DAY in your workflow!**
    TEV site is cleaved in 2 to 4h instead of 8h or overnight with a standard TEV Protease.
  - **Efficient in a wide range of buffers and temperatures**
  - **Able to cleave immobilized targets from affinity column**
  - **Removed efficiently from a reaction with IMAC resin**
- **Cleavage efficiency of the TEV site is target dependent.**
- **Best practice: Screen for optimal conditions (amount of protease and/or reaction time) with your own target:**
  - Mix 15 µg of target for 1 µL (10 U) of Cold-Active TEV Protease in the 1X reaction buffer
  - Incubate at 4°C or 20°C
  - Harvest samples at various times and analyze cleavage efficiency of your target

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