

# Numacut™ TEV protease: Revolutionizing Scarless Peptide and Protein Production

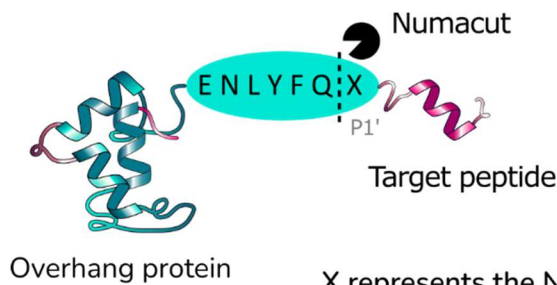


## Introducing Numacut TEV protease

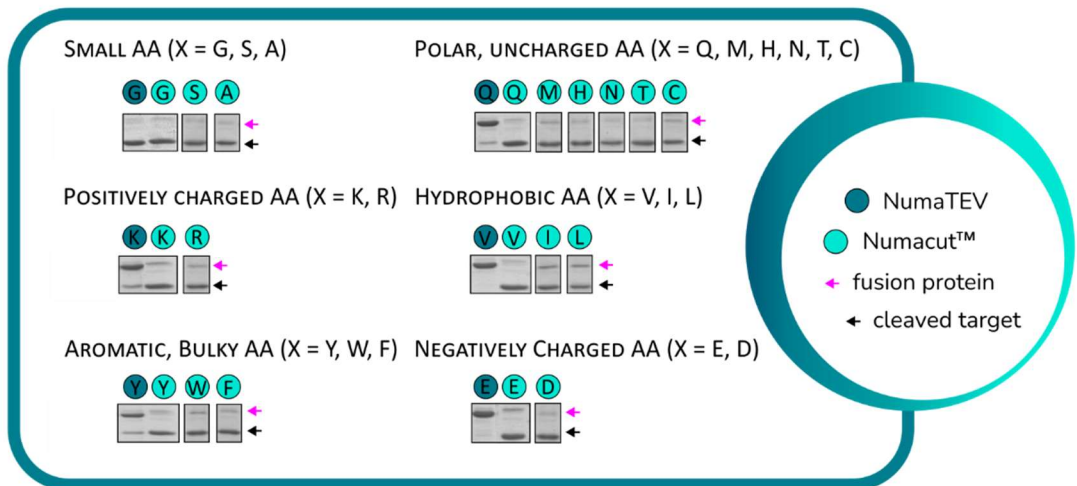
The Numacut TEV protease, developed through artificial intelligence, directed evolution, and rational design approaches represents an innovative advancement in the biotechnological field. In contrast to typical TEV proteases, Numacut displays enhanced substrate tolerance, accommodating 19 of the 20 standard amino acids (excluding P) at the P1' position. This unique feature facilitates the production of scarless peptides and proteins, positioning Numacut as the first and only protease platform designed for this purpose.

## Superior Functionality

Extensive research, including the development and testing of 20 identical fusion proteins varying only in the amino acid at the P1' position, has demonstrated Numacut's exceptional substrate tolerance (**Figure 1**). In comparative tests between the in-house wildtype TEV protease ("NumaTEV") and the newly developed Numacut TEV protease, Numacut exhibited cleavage across a wide range of amino acids, including those where the wildtype shows limited or no cleavage activity for these same amino acids.



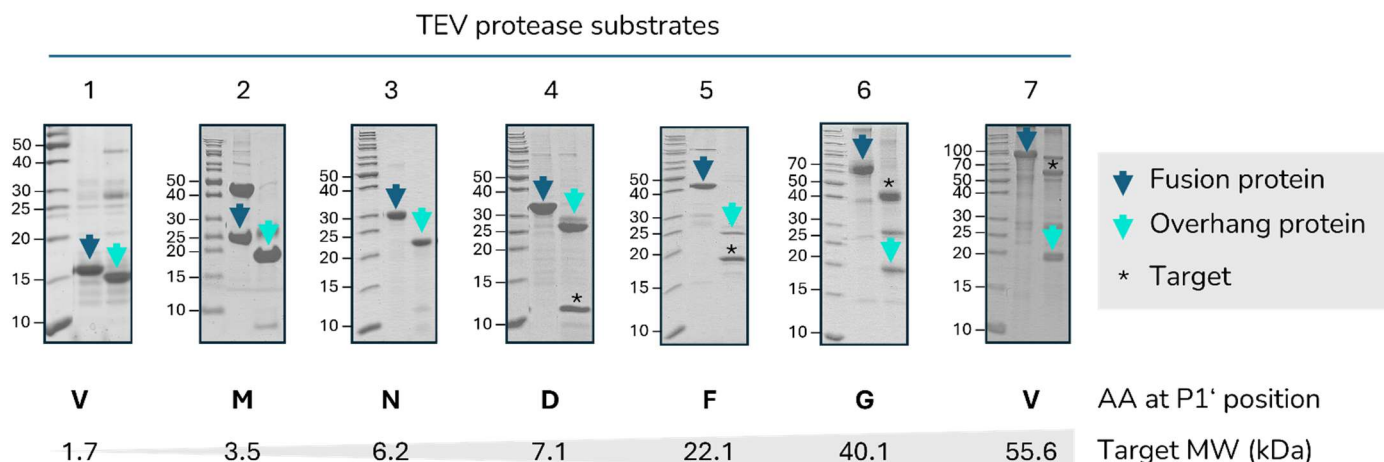
X represents the N-terminal amino acid (AA) of your target



Note: For X = P no cleavage was observed for all TEV protease variants

**Figure 1: Comparative Cleavage Efficiency of Numacut TEV Protease and Wildtype TEV Protease.** This figure presents the results of a cleavage assay designed to demonstrate the enhanced substrate tolerance of the Numacut TEV protease compared to Numaferm's wildtype TEV protease ("NumaTEV"). To conduct this study, 20 fusion proteins were engineered, each incorporating the TEV protease recognition site ENLYFQ and 1 of the 20 canonical amino acids at P1' between a fusion protein and a target peptide (4 kDa), respectively. The assay involved adding equal units of Numacut TEV protease (light blue) and NumaTEV (blue) to 3 µg of each test substrate. These mixtures were then incubated in reaction buffer (50 mM Tris-HCl pH 8, 0.5 mM EDTA, and 1 mM DTT) at 30°C for 3 hours. Post-incubation, the cleavage reactions were analyzed via 15% SDS-PAGE gels, with results visualized through Coomassie blue staining.

Furthermore, the exceptional performance of the Numacut TEV protease is further demonstrated by its ability to efficiently release target proteins of varying sizes and properties without producing any side products by non-specific cleavage, thereby maintaining high specificity even with longer reaction time (**Figure 2**).



**Figure 2: Impact of Target Size and Nature on Cleavage Efficiency by Numacut TEV Protease.** This figure showcases the results of an experiment designed to evaluate the impact of target protein size and properties on the cleavage efficiency of Numacut TEV protease. To achieve this, seven distinct fusion proteins were designed, each with targets varying in molecular weight from 1.7 to 55.6 kDa. These targets were positioned immediately following the newly optimized Numacut TEV protease recognition site, ENLYFQ. In the depicted assay, identical experimental conditions were employed as outlined in Figure 1, with Numacut TEV protease being added to each fusion protein variant. The reaction was facilitated in the same buffer system and incubated under identical conditions to ensure consistency and reliability in the outcomes.

### Benefits of Numacut TEV protease

- **Maximized Outcomes:** Numacut's ability to produce authentic, scarless proteins ensures that the solubility, stability, and functionality profiles of your targets are unaffected by overhangs, optimizing the results of your research.
- **Ease of Use:** The plug-and-play nature of Numacut TEV protease simplifies the cloning process, allowing for consistent use across different projects.
- **Regulatory Compliance:** Numacut alleviates regulatory concerns by avoiding the addition of extra amino acids, producing sequences that match those found in nature.
- **High Activity:** Engineered for high activity, Numacut ensures a reliable supply chain capable of supporting both R&D and GMP applications, from micrograms to kilograms.
- **Sustainability:** A unique lyophilized formulation extends the shelf life of Numacut TEV protease, supports room temperature shipping, and offers a sustainable alternative to wildtype variants.

### Conclusion

Numacut TEV protease is not just an enzyme; it's a transformative solution for the scarless production of peptides and proteins. Its exceptional specificity, combined with a broad substrate tolerance, simplifies the production process and assures complete cleavage no matter the condition. By choosing Numacut, researchers and manufacturers can confidently meet their needs for precision and efficiency in protein production. For further information, please do not hesitate to reach out to us. Find our contact details below.

Numaferm GmbH • Merowingerplatz 1a • 40225 Düsseldorf • Germany

Phone: +49 0 211 97532900 • E-Mail: [Products@numaferm.com](mailto:Products@numaferm.com)

<https://numaferm.com/products/numacut-tev-protease/>