Product Information

# Thrombin Protease, Biotin-tagged

Human recombinant, expressed in HEK 293 cells, ≥5,000 units/mL

**SAE0147**

CAS RN 9002-04-4

E.C. 3.4.21.5

Synonyms: Factor IIa, Fibrinogenase, Thrombase

## Product Description

Thrombin is an endolytic serine protease that selectively cleaves the Arg-Gly bonds of fibrinogen to form fibrin and release fibrinopeptides A and B.[1,2] The optimal cleavage sites for thrombin are as follows:[1]

- A-B-Pro-Arg-||-X-Y, where A and B are hydrophobic amino acids, and X and Y are nonacidic amino acids.
- Gly-Arg-||-Gly

Recombinant human thrombin protease is expressed in human HEK 293 cells as a glycoprotein heterodimer. The DTT-reduced protein migrates as two bands of ~31 kDa (heavy chain) and ~6 kDa (light chain) on SDS-PAGE. This protein is manufactured in human cells, with no serum. The human cells expression system allows human-like glycosylation and folding, and often supports higher activity of the protein. The protein is produced with no artificial tags.

This thrombin protease is useful for cleaving recombinant proteins that are expressed as fusion proteins with this sequence between the carrier domain and the protein of interest.

This biotinylated thrombin protease can be used for on-column cleavage of fusion proteins with a thrombin cleavage site. It specifically cleaves the protein of interest from a column-bound fusion protein, leaving the fusion domain or tag bound to the affinity column (For example, Ni-NTA column) and eluting only the protein of interest. This method is advantageous over post-elution cleavage for several reasons:

- It eliminates most impurities normally associated with purification on Ni-chelating columns.
- It allows gentler elution conditions, with added flexibility in the elution buffer composition. This can mitigate protein aggregation and inactivation.

After cleavage, the protease can be removed with any avidin-conjugated or streptavidin-conjugated beads.

The product is supplied in an aqueous buffer of pH 6.0, with 50% (v/v) glycerol.

Catalytic pH range: 5-10

Optimal pH: 8.3

**Note:** Thrombin precipitates at pH ≤5

Molecular mass: 37.4 kDa

Human isozymes pI range: 6.35-7.6

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage and Stability

The product is stable for at least two years as supplied. After opening, it is recommended to store the remaining protein in working aliquots at -20 °C. Since thrombin solutions adsorb to glass,[3] it is recommended to aliquot the solution in plastic tubes.

## Procedure

Thrombin protease is active over a wide range of temperatures (2-37 °C), ionic strength (0-400 mM NaCl), and pH (5-10). However, its activity may vary, depending on the substrate and conditions. It is therefore recommended to optimize the reaction conditions for each cleaved protein.

As a starting point, 10 units of thrombin protease can be used per 100 µg of target protein for 1 hour at 30 °C, or overnight at 2-8 °C. To improve cleavage, the addition of $CaCl_2$ is recommended (For example, 2.5-5 mM).

To perform on-column cleavage:

1. Dilute the desired amount of thrombin protease in a volume equal to one column volume.
2. Inject the protease solution directly into the column.
3. Incubate the column at the desired temperature and time according to the guidelines described above.
4. Elute the cleaved target protein with 1-3 column volumes, depending on the required protein concentration.
5. If the target protein is prone to precipitation at higher concentrations, elution can be performed by continually circulating the protease solution in a larger volume through the column in a closed circle, until all target protein is removed from the column.

**Unit definition**: One unit is defined as the amount of thrombin protease that cleaves 1 nmole of a chromogenic thrombin peptide in 1 minute, at room temperature, pH 8.0.

## References


1. Chang, J.-Y., Eur. J. Biochem., 151(2), 217-224 (1985).
2. Doolittle, R.F., in The Plasma Proteins, Volume II (Biosynthesis Metabolism, Alterations in Disease), 2nd ed. (Putnam, F.W., ed.). Academic Press (New York, NY), pp. 148-149 (1975).
3. Waugh, D.F. et al., J. Biomed. Mater. Res., 9(5), 511-536 (1975).


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