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Product Information

Thrombin human
BioUltra
recombinant, expressed in HEK 293 cells

Catalog Number **T9326**
Storage Temperature –70 °C

CAS RN 9002-04-4
EC 3.4.21.5
Synonyms: Factor IIa, FIIa, fibrinogenase, thrombase, tropostasin, activated blood-coagulation factor II
EXPASY/SwissProt P00734

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 predominant form of thrombin *in vivo* is the zymogen prothrombin (factor II), which is produced in the liver. The concentration of prothrombin in normal human plasma is 5–10 mg/dL.[2] Prothrombin is a glycoprotein with a glycan content of ~12%.[2] Prothrombin is cleaved *in vivo* by activated factor X (factor Xa), releasing the activation peptide and cleaving thrombin into light and heavy chains, which yields catalytically active α-thrombin.

α-Thrombin is composed of a light chain (A chain, MW ~6 kDa) and a heavy chain (B chain, MW ~31 kDa). These two chains are joined by one disulfide bond. The B chain of human thrombin consists of a peptide portion (MW 29,485 Da) and a carbohydrate portion (MW 2,334 Da) with N-linked glycosylation at three Asn residues.[3,4] Human thrombin has been reported to contain 4.1% hexose, 1.7% sialic acid, and ~2.4% acetylglucosamine.[5,6]

Autolytic degradation of α-thrombin results in the formation of β- and γ-thrombin. These catalyze cleavage of chromogenic synthetic substrates, but have lower fibrinogen clotting activity. β-Thrombin is formed from α-thrombin by degradation of the A chain and the excision of a small fragment containing a carbohydrate from the B chain.[5]

Thrombin also contains γ-carboxyglutamyl residues. These modified glutamyl residues are the result of carboxylation by vitamin K-dependent carboxylase, a microsomal enzyme. γ-Carboxyglutamyl residues are necessary for the Ca^{2+}-dependent interaction with a negatively charged phospholipid surface, which is essential for the conversion of prothrombin to thrombin.

Prothrombin is activated *in vivo* on the surface of a phospholipid membrane that binds the N-terminus of prothrombin along with factors Va and Xa. The activation process starts slowly because factor V is activated to factor Va by the initial small amount of thrombin.

The optimal cleavage sites for thrombin are as follows:[1]
1. A-B-Pro-Arg-||-X-Y, where A and B are hydrophobic amino acids, and X and Y are nonacidic amino acids
2. Gly-Arg-||-Gly

Thrombin cleavage of fibrinogen occurs only at Arg residues. However, the cleavage is not site-specific, and generally results in 2 products:
- The primary cleavage product, fibrinopeptide A, is cleaved from fibrinogen after amino acid 16 and sometimes after amino acid 19.
- A secondary cleavage product, fibrinopeptide B, is produced by cleavage at amino acid 14.[7]

Thrombin from any mammalian species will clot the fibrinogen of any other mammalian species.[2] Thrombin does not require divalent metal ions or cofactors for activity. However, Na^+-dependent allosteric activation of thrombin has been shown to play a role in defining the primary specificity of thrombin to cleave after Arg residues.[8]

Thrombomodulin serves as a cofactor for thrombin during the activation of protein C.[9] Thrombin will catalyze the hydrolysis of several peptide *p*-nitroanilides, tosyl-Arg-nitrobenzyl ester, and thiobenzyl ester synthetic substrates.[10]

Catalytic pH range:[11] 5–10
Optimal pH:[11] 8.3
(Note: thrombin precipitates at pH ≤5)

Molecular mass:[4,12] 37.4 kDa
Human isozymes pI range: 6.35–7.6
$E_{280}^{1\%}$ = 18.3 (human)[12]

Thrombin can also be used to cleave fusion proteins. Fusion protein cleavage can be performed at a thrombin:fusion protein ratio of 1:500.[13] A concentration of 0.5 NIH units thrombin per one nanomole of polypeptide in 20 µL of 50 mM ammonium bicarbonate, pH 8.0, has also been described.[1]

This product is supplied as a solution in 20 mM MES, pH 6.0, and 500 mM choline chloride.

Protein concentration:
≥0.10 mg protein/mL (UV, $E_{280}^{1\%}$ = 18.3)

Enzymatic Activity:
≥2,000 NIH units/mg protein ($E_{280}^{1\%}$ = 18.3)

Unit Definition: Activity is expressed in NIH units, and is measured by direct comparison to an NIH Thrombin Reference Standard. The NIH is based on a modification of the method of Biggs.[14] Only clotting times in the range of 15–25 seconds are used for determining thrombin activity. The optimal clotting temperature is 37 °C.

Thrombin concentrations in the literature are typically reported in terms of different units of activity.[14,15] Several conventions are used to express thrombin activity in the literature:

1 IOWA unit = 0.83 NIH unit
1 WHO unit = 1 NIH unit
1 NIH unit = 0.324 ± 0.073 µg
1 NIH unit = 1 USP unit

This recombinant human thrombin product 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 product 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 recombinant tags.

Precautions and Disclaimer
This product is 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/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 –70 °C. Since thrombin solutions adsorb to glass, it is recommended to aliquot the solution in plastic tubes.

References

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RBG,GCY,TMG,RXR,NA,MAM 06/18-1