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Product Information

Thrombin
from human plasma

Catalog Number **T6884**
Storage Temperature –20 °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.[3]

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.[10] 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 (human and bovine) 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. Cleavage of fusion proteins can be carried out 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.[2]

This product is lyophilized from a solution of saline sodium citrate buffer, pH 6.5.

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

Unit Definition: Activity is expressed in NIH units obtained by direct comparison to an NIH Thrombin Reference Standard. The NIH assay procedure uses 0.2 mL of diluted plasma (1:1 with saline) as a substrate and 0.1 mL of albumin solution based on a modification of the method of Biggs.[13] Only clotting times in the range of 15–25 seconds are used for determining thrombin activity. 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

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.

Preparation Instructions
The product is soluble in water (10 mg/mL).

Storage/Stability
Store the lyophilized powder at –20 °C. The product retains activity for at least 5 years.

Stock solutions can be prepared at a concentration of 100 units/mL in a 0.1% (w/v) BSA solution. Stock solutions remain active for one week at 0–5 °C. Solutions are most stable at pH 6.5, as a pH >7 will greatly reduce thrombin activity. Since thrombin solutions adsorb to glass, it is recommended to aliquot the solutions in plastic tubes and store at –20 °C for long-term storage.

References

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RBG,TMG,RXR,GCY,MAM 12/17-1