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Product Information

Thrombin from bovine plasma

Catalog Number **T6200**
Storage Temperature –20 °C

CAS RN 9002-04-4
EC 3.4.21.5
Synonym: Factor IIa

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.[3] Prothrombin is a glycoprotein with a glycan content of ~12%.[3]

Prothrombin is cleaved *in vivo* by activated factor X, releasing the activation peptide and cleaving thrombin into light and heavy chains yielding 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.[4] 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.[5,6] Bovine thrombin contains 1.7% glucosamine, 1.8% sialic acid, 0.61% galactose, and 0.95% mannose.[7]

Thrombin also contains γ-carboxyglutamyl residues. These modified glutamyl residues are the result of carboxylation by a microsomal enzyme, vitamin K-dependent carboxylase. 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.[4] Prothrombin is activated *in vivo* on the surface of a phospholipid membrane that binds the amino 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.

Optimal cleavage sites for thrombin:[2]
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 from any mammalian species will clot the fibrinogen of any other mammalian species.[8]

Thrombin cleavage of fibrinogen occurs only at Arg residues; however, the cleavage site is not specific, resulting in 2 products. The primary cleavage product, fibrinopeptide A, is cleaved from fibrinogen after amino acid 16 and sometimes after amino acid 19, while a secondary cleavage product, fibrinopeptide B is produced by cleavage at amino acid 14.[9]

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.[10] Thrombomodulin serves as a cofactor for thrombin during the activation of protein C.[11]

Under certain storage conditions, autolytic digestion of α-thrombin results in formation of β and γ-thrombins, which lack fibrinolytic activity, but retain some activity against synthetic peptide substrates and protein substrates other than fibrinogen.[12] This thrombin preparation is predominantly α-thrombin.

Thrombin (human and bovine) will catalyze the hydrolysis of several peptide *p*-nitroanilides, tosyl-Arg-nitrobenzyl ester, and thiobenzyl ester synthetic substrates.[13]

Catalytic pH range:[14] 5–10, optimal pH:[14] 8.3
thrombin precipitates ≤pH 5

Human isozymes pI range: 6.35–7.6.
Bovine pI range:[15] 7.05–7.1

$E_{280}^{1\%}$ = 18.3 (human)[16]

$E_{280}^{1\%}$ = 19.5 (bovine)[17]

This product is lyophilized from a solution containing sodium chloride and Tris-HCl, pH 7.0. It is activated with bovine lung thromboplastin.

Specific Activity: 40–300 NIH units/mg protein
($E_{280}^{1\%}$ = 19.5)

Unit Definition: Activity is expressed in NIH units obtained by direct comparison to a NIH Thrombin Reference Standard, Lot K. 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.[18] 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.[18,19] 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
The product is soluble in water (10 mg/ml), yielding a clear solution.

Storage/Stability
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.

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

Related Products
Synthetic Substrates:[13]
N-Benzoyl-Phe-Val-Arg-*p*-nitroanilide
(Catalog Number 13042)
N-Benzoyl-Phe-Val-Arg-*p*-nitroanilide hydrochloride
(Catalog Number B7632)
N-Benzoyl-Phe-Val-Arg 4-methoxy-β-naphthylamide
(Catalog Number B1260)
Boc-β-benzyl-Asp-Pro-Arg-7-amido-4-methylcoumarin
(Catalog Number B4028)
Boc-Val-Pro-Arg-7-amido-4-methylcoumarin
(Catalog Number B9385)
Sar-Pro-Arg *p*-nitroanilide
(Catalog Number S9009)
Thrombin generation chromogenic substrate
(Catalog Number T3068)
N-*p*-Tosyl-Gly-Pro-Arg 7-amido-4-methylcoumarin
(Catalog Number T0273)
N-(*p*-Tosyl)-Gly-Pro-Arg *p*-nitroanilide
(Catalog Number T1637)

Inhibitors:[20-22]
Diisopropylfluorophosphate
(Catalog Number D0879)
Phenylmethylsulfonyl fluoride
(Catalog Number P7626)
AEBSF
(Catalog Number A8456)
Hirudin
(Catalog Number H7016)
Proflavine
(Catalog Number P2508)
Antithrombin III
(Catalog Number A2221)
α_1-antitrypsin
(Catalog Number A9024)
α_1-antiplasmin
(Catalog Number A8849)
Gabexate mesylate
(Catalog Number G2417)
Antipain
(Catalog Number A6191)
N_α-Tosyl-L-lysine chloromethyl ketone hydrochloride
(Catalog Number T7254)

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   See also: The Enzyme Explorer: Plasma and Blood Protein Resource
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