

## Product Information

### Thrombin from human plasma

Catalog Number **T7009**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

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.<sup>1,2</sup>

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.<sup>3</sup> Prothrombin is a glycoprotein with a glycan content of ~12%.<sup>3</sup>

Prothrombin is cleaved *in vivo* by activated factor X (factor Xa) releasing the activation peptide and cleaving thrombin into light and heavy chains yielding catalytically active  $\alpha$ -thrombin.  $\alpha$ -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.<sup>4</sup> 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.<sup>5,6</sup> Bovine thrombin contains 1.7% glucosamine, 1.8% sialic acid, 0.61% galactose, and 0.95% mannose.<sup>7</sup>

Autolytic degradation of  $\alpha$ -thrombin results in the formation of  $\beta$ - and  $\gamma$ -thrombin, which catalyze cleavage of chromogenic, synthetic substrates, but have lower fibrinogen clotting activity.  $\beta$ -Thrombin is formed from  $\alpha$ -thrombin by degradation of the A chain and the excision of a small fragment containing a carbohydrate from the B chain.<sup>5</sup>

Thrombin also contains  $\gamma$ -carboxyglutamyl residues. These modified glutamyl residues are the result of carboxylation by a microsomal enzyme, vitamin K-dependent carboxylase.  $\gamma$ -Carboxyglutamyl residues are necessary for the  $\text{Ca}^{2+}$ -dependent interaction with a negatively charged phospholipid surface, which is essential for the conversion of prothrombin to thrombin.<sup>4</sup>

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.

Optimal cleavage sites for thrombin:<sup>2</sup>

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 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.<sup>8</sup>

Thrombin from any mammalian species will clot the fibrinogen of any other mammalian species.<sup>9</sup>

Thrombin does not require divalent metal ions or cofactors for activity. However,  $\text{Na}^{+}$ -dependent allosteric activation of thrombin has been shown to play a role in defining the primary specificity of thrombin to cleave after Arg residues.<sup>10</sup> Thrombomodulin serves as a cofactor for thrombin during the activation of protein C.<sup>11</sup>

Thrombin (human and bovine) will catalyze the hydrolysis of several peptide *p*-nitroanilides, tosyl-Arg-nitrobenzyl ester, and thiobenzyl ester synthetic substrates.<sup>12</sup>

Catalytic pH range:<sup>13</sup> 5–10, optimal pH:<sup>13</sup> 8.3  
thrombin precipitates  $\leq\text{pH } 5$

Molecular mass:<sup>6,14</sup> 37.4 kDa

Human isozymes pI range: 6.35–7.6.

$E_{280}^{1\%} = 18.3$  (human)<sup>14</sup>

Thrombin can also be used to cleave fusion proteins. Cleavage of fusion proteins can be carried out at a thrombin to fusion protein ratio of 1:500.<sup>15</sup> A concentration of 0.5 NIH units thrombin per one nanomole of polypeptide in 20  $\mu$ l of 50 mM ammonium bicarbonate, pH 8.0, has also been described.<sup>2</sup>

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

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

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.<sup>16</sup> 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.<sup>16,17</sup> 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 =  $0.324 \pm 0.073$   $\mu$ g
- 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).

### 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.

### Related Products

#### Synthetic Substrates:<sup>12</sup>

- 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- $\beta$ -naphthylamide  
(Catalog Number B1260)
- Boc- $\beta$ -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:<sup>18-20</sup>

- 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)
- $\alpha_1$ -antitrypsin  
(Catalog Number A9024)
- $\alpha_1$ -antiplasmin  
(Catalog Number A8849)
- Gabexate mesylate  
(Catalog Number G2417)
- Antipain  
(Catalog Number A6191)
- $N_\alpha$ -Tosyl-L-lysine chloromethyl ketone hydrochloride  
(Catalog Number T7254)

## References

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