Plasma Derived Proteins and Enzymes
Coagulation Pathway Proteins

Fibrinopeptide A human

| Alad-Ser-Gly-Gly-Gly-Phe-Leu-Ala-Gly-Gly-Val-Arg |
| C<sub>68</sub>H<sub>97</sub>N<sub>19</sub>O<sub>26</sub> FW 1536.56<br>≥97% (HPLC) |
Fibrin polymerization is initiated by thrombin cleavage of fibrinopeptide A from fibrinogen α chains, exposing two E domain E(A) sites. The phenylalanine at α chain residue 8 is important for efficient thrombin-catalyzed proteolysis of fibrinogen. Substitution of tyrosine for phenylalanine blocks sequential fibrinopeptide release and, thus, blocks polymerization and clot formation.

Fibrinopeptide B human

| pGlu-Gly-Val-Asn-Asp-Asn-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg |
| C<sub>66</sub>H<sub>93</sub>N<sub>19</sub>O<sub>25</sub> FW 1552.56<br>≥97% (HPLC) |
Sequential release of fibrinopeptides by thrombin action on fibrinogen is essential to normal fibrin polymerization. Cleavage of fibrinopeptide B from fibrinogen B β chains exposes E domain polymerization sites, E(B), that interact with platelets, fibroblasts, and endothelial cells.

[Glu]<sup>1</sup>-Fibrinopeptide B human

| Glu-Gly-Val-Asn-Asp-Asn-Glu-Gly-Phe-Ser-Ala-Arg |
| C<sub>66</sub>H<sub>95</sub>N<sub>19</sub>O<sub>26</sub> FW 1570.57<br>≥90% (HPLC) |
Indicator of coagulation and fibrinolytic activity

Hirudin from leeches

**[Lys]<sup>57</sup>Hirudin (HV2), leech**

recombinant, expressed in *Saccharomyces cerevisiae*, activity: ≥7,000 ATU/mg protein (ATU = antithrombin units)
One unit (ATU) will neutralize one NIH unit of thrombin at 37 °C, based on direct comparison to an NIH thrombin reference standard.

Protein determined by Lowry.
S: 22-24/25

Kistrin from *Agkistrodon rhodostoma* (Malayan pit viper) venom

lyophilized powder, γ-irradiated, 95% (SDS-PAGE)
Disintegrins represent a novel family of integrin β1 and β3 inhibitor proteins isolated from viper venoms. They are low molecular-weight, cysteine-rich peptides containing the Arg-Gly-Asp (RGD) sequence. They are the most potent known inhibitors of integrin function. Disintegrins interfere with cell adhesion to the extracellular matrix, including adhesion of melanoma cells and fibroblasts to fibronectin, and are potent inhibitors of platelet aggregation.

Plasma barium sulfate eluate from bovine plasma

lyophilized powder
Contains prothrombin and factors VII, IX and X.
One unit is equivalent to the eluate from one liter of plasma.

Hiridin, see under Renin-Angiotensin System Page 20
Kininogen, see under Renin-Angiotensin System Page 20
Plasminogen

Proteinase: [9001-91-6]

Plasminogen is the inactive precursor of the protease plasmin. Plasminogen is activated by the action of either tissue plasminogen activator (tPA), which primarily activates the fibrinolytic (thrombolytic) activity of plasmin, or urokinase plasminogen activator (uPA), which is associated with extracellular matrix remodeling and cell migration. Plasmin cleaves fibrin/fibrinogen and blood coagulation factors Va and VIIIa. It activates matrix metalloproteinases by cleaving the inactive proenzymes. It is also involved in the activation of some growth factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β).

One unit will produce a ΔA235 of 1.0 from α-casein in 20 min at pH 7.5 at 37 °C, when measuring perichloric acid soluble products in a volume of 5.0 mL. Activity determined after activation to plasmin with urokinase.

Plasminogen from bovine plasma

Lyophilized powder, activity: 3-5 units/mg protein
- e-Aminocaproic acid free
- Lyophilized powder containing NaCl, EDTA, lysine, and Tris buffer salts not suitable for streptokinase determination by clot formation procedure
- Composition
  - Protein ~5% (biuret)
- Solutions should be stored in buffer containing 20 mM lysine at neutral pH. The protein should be as concentrated as possible. Frozen in aliquots at −20 °C, it should be stable for several weeks.

EC No. 232-641-9 [SR1]

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Plasminogen from human plasma

EC No. 2326419

Lyophilized powder containing NaCl, EDTA, lysine, and Tris buffer mol wt 90 kDa
- Solutions should be stored in buffer containing 20 mM lysine at neutral pH. The protein should be as concentrated as possible. Frozen in aliquots at −20 °C, it should be stable for several weeks.
- Plasma from each donor has been tested and found negative for antibody to HIV-1/HIV-2, antibody to HCV and HbsAg.
- Lyophilized powder, activity: ≥2 units/mg protein, suitable for streptokinase determination by clot formation procedure
- Composition
  - Protein ~5% (biuret)

Composition: <0.005 unit/Unit plasminogen

EC No. 232-641-8 [SR1]

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Plasma and Blood Related Proteins

10 Proteins

Plasma and Blood Derived Proteins

Plasminogen (continued)

- Lyophilized powder, activity: ≥2 units/mg protein
  - not suitable for streptokinase determination by clot formation procedure
  - plasmin ................................................................. <0.2 unit/unit plasminogen
    P7397-SUN 5 units
    P7397-10UN 10 units
    P7397-25UN 25 units

Plasminogen from rabbit plasma

Lyophilized powder, activity: 4-10 units/mg protein
- Contains NaCl, EDTA, lysine and Tris buffer
- not suitable for streptokinase determination by clot formation procedure
- composition
  - Protein ~5% (biuret)
- Prepare solution in buffer containing 20 mM lysine at neutral pH. The protein should be as concentrated as possible. Frozen in aliquots at −20 °C, it should be stable for several weeks.
- Aminocaproic acid free
- Composition:
  - plasmin ................................................................. <0.01 unit/unit plasminogen
  - EC No. 232-641-9
  - P2284-SUN 5 units

Protein C

Protein C from human plasma

[60202-16-6]
- Lyophilized powder
- Zymogen
  - Protein C is a plasma, vitamin K-dependent zymogen of a serine protease that can inhibit blood coagulation by inhibiting thrombin formation, selectively inactivating factors Va and Villa. The Protein C anticoagulant pathway is triggered when thrombin binds to the endothelial cell proteoglycan, thrombomodulin. This complex, which cannot clot blood, is a potent activator of the protein C zymogen.
  - Activation involves the release of a dodecapeptide from the N-terminal domain of the heavy chain. The activated Protein C (APC) then binds to protein S on cell surfaces and inactivates the coagulation factors Va and Villa by proteolysis. APC has also been shown to bind to receptors on the endothelium of large blood vessels.
  - Lyophilized powder from 20 mM Tris-HCl, pH 7.4, containing 0.1 M NaCl
  - light chain mol wt 21 kDa
  - heavy chain mol wt 41 kDa
  - solubility
    - H2O ................................................................. 1 mg/mL
    - ≥36°C
    - P2200-.1MG 0.1 mg
  - Composition:
    - Protein ~5% (biuret)
    - Aminocaproic acid free

Protein C from human plasma

Activated Protein C
[42617-41-4] E.C. 3.4.21.69
- Activated, lyophilized powder, ≥90% (SDS-PAGE)
  - Protein C is a plasma, vitamin K-dependent zymogen of a serine protease that can inhibit blood coagulation by inhibiting thrombin formation, selectively inactivating Factors Va and Villa. The Protein C anticoagulant pathway is triggered when thrombin binds to the endothelial cell proteoglycan, thrombomodulin. This complex, which cannot clot blood, is a potent activator of the protein C zymogen.
  - Activation involves the release of a dodecapeptide from the N-terminal domain of the heavy chain. The activated Protein C (APC) then binds to protein S on cell surfaces and inactivates the coagulation factors Va and Villa by proteolysis. APC has also been shown to bind to receptors on the endothelium of large blood vessels.
  - Lyophilized powder from 20 mM Tris-HCl, pH 7.4, containing 0.1 M NaCl
  - light chain mol wt 21 kDa
  - heavy chain mol wt 41 kDa
  - solubility
    - H2O ................................................................. 1 mg/mL
    - ≥36°C
    - P2200-.1MG 0.1 mg
  - Composition:
    - Protein ~5% (biuret)
    - Aminocaproic acid free

Protein Z from human plasma

Aqueous glycerol solution
- Required for binding of thrombin to endothelial phospholipids. May be involved in blood coagulation.
- Solution in 50% glycerol in water;
  - mol wt 62 kDa
  - 10 units/mg solid
  - Composition:
    - Protein ~4%
Thrombin is an endolytic serine protease that selectively cleaves the Arg–Gly bonds of fibrinogen to form fibrin and release fibrinopeptides A and B. 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 ~31,000. Prothrombin is activated 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 itself has to be activated by the initial small amounts of thrombin. The optimal cleavage sites for thrombin have been determined to be 1) A-B-Pro-Arg-[II]-X-Y where A and B are hydrophobic amino acids and X and Y are nonacidic amino acids and 2) Gly-Arg-[II]-Gly.

Thrombin from any mammalian species will clot the fibrinogen of any other mammalian species. Thrombin is active in the pH range of 5-10. Catalytic optimum is pH 8.3. Human thrombin consists of several isoforms with isoelectric points in the range of 6.35-7.6. For bovine the pl range is 7.05 - 7.114. Typically one to two units of thrombin will clot one mL of plasma.

**Cleavage of Fusion Proteins:**

Thrombin can be used for the cleavage of many peptides at the thrombin recognition site using concentrations of 0.5 NIH units thrombin per one nanomole polypeptide in 20 microliters of 50 mM ammonium bicarbonate, pH 8.0. Thrombin cleavage of fusion proteins can be carried out at a thrombin to fusion protein ratio of 1:500. Fusion proteins may be cleaved in thrombin cleavage buffer consisting of 50 mM Tris, pH 8.0, 150 mM NaCl, 2.5 mM CaCl2 and 0.1% 2-mercaptoethanol. 2 mg of fusion protein can be incubated with 4 µg of thrombin for 20 minutes at RT in the cleavage buffer.

**Thrombin**

Factor IIa


**Thrombin from human plasma**

EC No. 2326487

Activity is expressed in NIH units obtained by direct comparison to a 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 thrombin sample (stabilized in 1% buffered albumin solution) based on a modification of the method of Biggs. Only clotting times in the range of 15-25 seconds are used for determining thrombin activity.

HIV and HBsAg ................................................ source material tested negative

**Lyophilized powder, activity: ≥2,000 NIH units/mg protein**

(EI\(^\circ\) = 18.3)

Lyophilized from saline sodium citrate buffer, pH 6.5

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**Lyophilized powder, activity: ~1,000 NIH units/mg protein**

(EI\(^\circ\) = 18.3)

Lyophilized from saline sodium citrate buffer, pH 6.5

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**Lyophilized powder, activity: >2800 NIH units/mg protein**

(EI\(^\circ\) = 18.3)

Lyophilized from 0.02 M Bis/Tris buffer, pH 6.5, 0.15 M NaCl and 0.1% PEG-8000

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**Thrombin at pH 7.5 at 37 °C in 10 min.**

One unit will liquify a standard clot of fibrinogen, plasminogen, and thrombin at pH 7.5 at 37 °C in 10 min.
Plasma Derived Proteins and Enzymes
Coagulation Pathway Proteins

Thrombin (continued)

- [ ] lyophilized powder, Suitable for routine use in the thrombin time test
  - vial of ≥10 NIH units
  - EC No. 232-648-7
  - RTECS # X08950000
  - T8885-10VL 10 vials
  - T8885-25VL 25 vials

- [ ] lyophilized powder, Suitable for routine use in the thrombin time test
  - contains bovine albumin as stabilizer
  - vial of ≥10 NIH units
  - Prepared from Product No. T7009.
  - T9135

- [ ] lyophilized powder, activity: 50-300 NIH units/mg protein
  - EC No. 36/37/38-42: S. 22-24-26-36/37
  - RTECS # X08950000
  - T9677

- [ ] lyophilized powder, activity: 50-300 NIH units/mg protein
  - Lyophilized powder containing sodium chloride and Tris-HCl
  - EC No. 36/37/38-42: S. 22-24-26-36/37
  - RTECS # X08950000
  - T0553

Thrombin from bovine plasma

EC No. 2326487
Activity is expressed in NIH units obtained by direct comparison to a NIH Thrombin Reference Standard, Lot J.

The NIH assay procedure uses 0.2 mL of diluted plasma (1:1 with saline) as a substrate and 0.1 mL of thrombin sample (stabilized in a 1% buffered albumin solution) based on a modification of the method of Biggs. Only clotting times in the range of 15-25 seconds are used for determining thrombin activity.

S: 22-24/25

- [ ] lyophilized powder, activity: 40-300 NIH units/mg protein (biuret)
  - Lyophilized powder containing sodium chloride and Tris-HCl, pH 7.0
  - Protein ~50%
  - Activated with bovine brain thromboplastin.
  - T4648-1KU 1,000 units
  - T4648-10KU 10,000 units

- [ ] lyophilized powder, activity: ≥2,000 NIH units/mg protein (biuret)
  - Lyophilized from saline sodium citrate buffer, pH 6.5
  - T7513-50UN 50 units
  - T7513-100UN 100 units
  - T7513-250UN 250 units
  - T7513-500UN 500 units

- [ ] lyophilized powder, activity: ≥0.5-150 NIH units/mg protein (Bradford)
  - Lyophilized powder containing sodium chloride and Tris-HCl
  - Protein ~50%
  - T3399-1KU 1,000 units
  - T3399-10KU 10,000 units

- [ ] lyophilized powder, activity: ≥700 NIH units/mg protein (Bradford)
  - Lyophilized from Tris buffer and sodium chloride
  - Protein ~25%
  - T9681-5KU 5,000 units

- [ ] lyophilized powder, activity: 175-350 NIH units/mg protein (biuret)
  - Lyophilized powder containing 75% sodium citrate, pH 5.8
  - Composition
  - Protein ~25%
  - T4265-500UN 500 units
  - T4265-2.5KU 2,500 units
  - T4265-10KU 10,000 units
  - T4265-25KU 25,000 units

- [ ] lyophilized powder, activity: ≥125 NIH units/mg protein (biuret)
  - In 0.05 M phosphate buffer, pH 7.0
  - Activated with bovine brain thromboplastin.
  - T9000-500UN 500 units
  - T9000-2.5KU 2,500 units

Thrombin from murine plasma

Lyophilized powder, activity: ~1,000 NIH units/mg protein (biuret)
Lyophilized from saline sodium citrate buffer, pH 6.5
Activity is expressed in NIH units obtained by direct comparison to a 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 thrombin sample (stabilized in a 1% buffered albumin solution) based on a modification of the method of Biggs. Only clotting times in the range of 15-25 seconds are used for determining thrombin activity.

S: 22-24/25

- [ ] lyophilized powder, activity: ≥125 NIH units/mg protein (biuret)
  - Lyophilized from saline sodium citrate buffer, pH 6.5
  - T8397-100UN 100 units

Thrombin ▲
**Factor II from human plasma**

Prothrombin

[9001-26-7]

Lyophilized powder, activity: 5-20 units/mg protein

Plasma phospholipid binding protein; binds to endothelial cells, promotes clotting on the cell surfaces.

When reconstituted with 1 mL of deionized water, solution will contain the indicated activity in 0.5 M NaCl, 0.05 M Tris-HCl, pH 8.0.

One unit is the amount contained in one mL of normal human plasma.

Thrombin .............................................................. none detected

Factor X ......................................................... <0.01 unit/unit of factor II

EC No. 232-592-3

FS12-10UN

10 units

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**Tissue plasminogen activator human**

pkg of 10 μg, activity: ≥500,000 IU/mg (activity expressed per mg of tPA)

Supplied as lyophilized powder containing 10 μg tPA lyophilized from a 100 μg/mL solution containing 1 M NH4HCO3 with 1% bovine serum albumin.

Tissue Plasminogen Activator (tPA) is a protease of the S1 family (trypsin family) and is found in a wide variety of mammalian tissues, especially endothelial cells. tPA is secreted as a single chain precursor, which is cleaved to a two-chain form by plasmin. Supplied in the single chain form with a molecular weight of ~70 kDa.

EC No. 232-917-9

RTECS # OB8900000

WET ICE

U9757

100 μg

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**Urokinase from human urine**

[9039-53-6] E.C. 3.4.21.73 EC No. 2329179

Lyophilized powder, activity: ≥500 units/mg protein

Lyophilized from 1 mL of 50 mM Tris-HCl, pH 7.4, with 100 mM NaCl, 0.1% PEG 6000, and 200 mM mannitol

One unit will activate that amount of porcine plasminogen which will produce a ΔA275 of 1.0 per minute at pH 7.5 at 37 °C, when measuring perchloric acid soluble products from α-casein (1 cm light path).

EC No. 232-917-9

RTECS # 08890000

WET ICE

U0633-50UG

50 μg

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**Urokinase-type Plasminogen Activator Receptor-1/Fc Chimera from mouse**

UPAR-1 recombinant, expressed in mouse NSO cells, >90% (SDS-PAGE), lyophilized powder

Chimeric protein receptor for uPA. The uPA/UPAR complex initiates activation of protein tyrosine kinases, gene expression, cell adhesion, and chemotaxis.

Mouse UPAR-1 (a.a. 1-297) fused to the C-terminal Fc region of human IgG1.

Lyophilized from a 0.2 μm filtered solution of phosphate buffered saline.

Calculated mol wt 56 kDa

Measured by its ability to bind human uPA.

S: 22-2405

WET ICE

U9757

100 μg

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Visit the Enzyme Explorer for Protease Detection

Kits & Reagents

[link](sigma-aldrich.com/enzymeexplorer)