



HIRUDIN

Product Number **H 7016, H 7380, H 9022, AND H 0393**

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

CAS #: 8001-27-2

Product Description

Molecular formula: 65-amino acid polypeptide with multiple isoforms.

Molecular weight:

Native:¹ 7050.7

Recombinant rHV2-Lys47:² 6906.5

Isoelectric point:³ 3.9

Hirudin is a potent specific inhibitor of thrombin produced in the salivary glands of the leech *Hirudo medicinalis*. At least 20 isoforms have been identified and sequenced.⁴

Hirudin inhibits blood coagulation by blocking the action of thrombin, thereby preventing the conversion of fibrinogen to fibrin. The hirudin- α -thrombin complex has a dissociation constant of approximately 1×10^{-11} M.^{3,5} The structure of the complex has been published.⁴

The amino acid sequence has been determined; it is a polypeptide of 65 amino acid residues containing a sulfated tyrosyl residue (#63) and three disulfide bridges.^{4,5} Hirudin is characterized by a high proportion of dicarboxylic acids, and by the absence of tryptophan, methionine, and arginine.

The method of preparation of hirudin from leeches is modified from Markwardt (1970).^{3,6} Hungry leeches, provide a richer source of hirudin.⁶ Product H 0393 is a recombinant protein, [Lys 47]-HV2 variant 1 produced by cDNA expressed in *Saccharomyces cerevisiae*.

Assay methods can be found in reference 3. Hirudin activity can be determined using standardized thrombin, and hirudin can be used to measure thrombin activity. The measurement of hirudin level in plasma by the ecarin clotting time assay can be affected by prothrombin deficiency.⁷

Sigma assays products H 7380, H 7016 and H 0393 by direct comparison to an NIH thrombin reference standard. Activity is expressed in anti-thrombin units

Product Information

(ATU).⁵ Product H 9022 is assayed using a fibrinogen assay.

Preparation Instructions

H 0393 and H 9022 give clear, colorless solutions at a concentration of 500 U/mL with water.

H 7380 and H 7016 are dissolved at a concentration of 500 U/mL of "Dilution Fluid II" (35.7 mM acetic acid, 35.7 mM sodium diethyl barbiturate, 0.85% NaCl, 1% bovine serum albumin, 0.5% PEG, pH 7.4).⁸

Hirudin is reported to be soluble in pyridine, but practically insoluble in alcohol, ether, acetone, or benzene.⁹

Storage/Stability

In dried form, hirudin was found to lose <5% of its activity in 2-3 years, when stored at $-20\text{ }^{\circ}\text{C}$.

In water with preservative added, it is stable for 6 months at room temperature. It is also stable when heated for 15 minutes at $80\text{ }^{\circ}\text{C}$. The heat stability decreases with increasing pH. It is stable for 15 minutes at $20\text{ }^{\circ}\text{C}$ in 0.1 N HCl or 0.1 N NaOH. Trypsin and α -chymotrypsin do not destroy the hirudin activity, probably due to the tertiary structure of the peptide (oxidized hirudin is readily destroyed by trypsin).³

References

1. Calculated from the amino acid sequence in Ref. 4
2. Van Dorssalaer, A., et al., *Biochemistry*, **28**, 2949 (1989)
3. Markwardt, F., *Methods in Enzymology*, **19**, 924-932 (1970).
4. Stone, S. R., and Maraganore, J. M., *Methods in Enzymology*, **223**, 312 (1993)
5. Dodt, J., et al., *FEBS Letters*, **165**, 180-184 (1984), citing Markwardt, F., and Walsmann, P., *Hoppe-Seyler's Z. Physiol. Chem.*, **312**, 85-98 (1958).
6. Bagdy, D., et al., *Methods in Enzymology*, **45**, 669-678 (1976).
7. Lindhoff-Last, E., et al., *Thromb. Res.*, **100**, 55 (2000).
8. Loison, G., et al., *Bio/Technology*, **6**, 72 (1988).
9. Merck Index, 11th ed., #4638 (1989).

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