



## RayBiotech, Inc.

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# Certificate of Analysis and Data Sheet Recombinant Hirudin

**Catalog No.**  
228-10710

**Source**  
*Pichia Pastoris*

## **Introduction**

Recombinant Hirudin is a potent thrombin inhibitor originally derived from the medicinal leech unlike heparin. Hirudin acts directly on thrombin rather than through other clotting factors. The mechanism of Hirudin-thrombin appears to be unique. The conversion of fibrinogen into fibrin by the serine protease enzyme thrombin is a major event in the final stages of blood coagulation. In the final stages of coagulation prothrombinase converts prothrombin into thrombin. Fibrin is subsequently cross linked by factor XIII to form a blood clot. The primary inhibitor of thrombin in normal blood circulation is antithrombin III. The anticoagulant activity of hirudin is derived from its ability to inhibit the pro-coagulant activity of thrombin (similar to antithrombin III activity). Hirudin is the strongest natural inhibitor of thrombin. Hirudin binds to and inhibits only the activity of thrombin forms with a specific activity on fibrinogen contrasting to antithrombin III activity. Therefore, hirudin has a thrombolytic activity since it prevents or dissolves the formation of clots and thrombi. Hirudin also has therapeutic significance in blood coagulation disorders, in the treatment of skin hematomas and of superficial varicose veins. Hirudin does not hinder with the biological activity of other serum proteins and can also act on complexed thrombin, thus having an advantage over more common anticoagulants and thrombolytics like heparin for example. It is complicated to extract large quantities of hirudin from natural sources; therefore a method for producing and purifying hirudin using recombinant biotechnology has been developed.

## **Description**

Recombinant Hirudin is derived from yeast and the polypeptide chain contains 65 amino acids and its Mw is 6979.5 dalton which is identical to natural Hirudin except for the substitution of leucine for isoleucine at the N-terminal end of the molecule and the absence of a sulfate group on the tyrosine at position 63. The Recombinant Hirudin is purified by proprietary chromatographic techniques.

## **Physical Appearance**

Sterile Filtered White lyophilized (freeze-dried) powder.

## **Formulation**

Each mg of protein was lyophilized from a sterile solution containing no 20mM PBS pH-7 and 2% mannitol.

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Not for diagnostic or therapeutic use.**



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### ***Purity***

Greater than 95.0% as determined by:

- (a) Analysis by RP-HPLC.
- (b) Analysis by SDS-PAGE.

### ***Solubility***

It is recommended to reconstitute the lyophilized Hirudin in sterile 18M $\Omega$ -cm H<sub>2</sub>O not less than 100 $\mu$ g/ml, which can then be further diluted to other aqueous solutions.

### ***Biological Activity***

The biological activity is measured using chromogenic assay, 1 unit is defined as the amount of Hirudin that neutralizes 1 unit of the WHO preparation 89/588 of thrombin. The specific activity was found to be 16,000ATU/mg protein.

### ***Stability***

Lyophilized Hirudin although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution Hirudin should be stored at 4°C between 2-7 days and for future use below -18°C. For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA).

**Please prevent freeze-thaw cycles.**

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