

## Product Information

### Hirudin from leeches

Catalog Numbers **H7016** and **H7380**

Storage Temperature 2–8 °C

CAS RN 8001-27-2

#### Product Description

The anticoagulant, hirudin, is the most potent natural inhibitor of both soluble and clot-bound thrombin. It binds thrombin with high affinity, the hirudin- $\alpha$ -thrombin complex has a dissociation constant of  $\sim 1 \times 10^{-11}$  M.<sup>1,2</sup> Hirudin covers more than 20% of the surface area of thrombin, occluding both the active site and exosite I (fibrinogen and PAR recognition site). This coverage blocks thrombus growth and platelet activation. Hirudin is not metabolized in the bloodstream of humans and is eliminated unchanged via kidney filtration.

Hirudin is a ~7 kDa acidic protein containing 65 amino acid residues. It contains a sulfated tyrosyl residue (Tyr<sup>63</sup>), three disulfide bridges, and a high proportion of dicarboxylic acids.<sup>2,3</sup> Hirudin is not glycosylated and lacks tryptophan, arginine, and methionine residues. At least 20 isoforms have been identified and sequenced.<sup>3</sup>

Isoelectric point (pI): 3.5–4.0.

Hirudin is produced in the salivary glands of the leech *Hirudo medicinalis*.<sup>4</sup> Hungry leeches provide a richer source of hirudin.<sup>4</sup> The method of preparation is a modification of published procedures.<sup>1,4</sup> Both products are supplied as lyophilized powders.

Specific Activity:

H7016,  $\geq 1,500$  antithrombin units/mg protein  
H7389, 300–1,500 antithrombin units/mg protein

One antithrombin unit (ATU) will neutralize one NIH unit of thrombin at 37 °C, based on direct comparison to an NIH thrombin reference standard.

#### 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

Hirudin is soluble in water. The literature cites the use of "Dilution Fluid II" (35.7 mM acetic acid, 35.7 mM sodium diethyl barbiturate, 0.85% NaCl, 1% bovine serum albumin, and 0.5% PEG, pH 7.4) to dissolve hirudin (500 ATU/mL) and thrombin.<sup>5</sup>

Hirudin is reported to be soluble in pyridine, but practically insoluble in alcohol, ether, acetone, or benzene.<sup>6</sup>

#### Storage/Stability

Store the products at 2–8 °C.

#### References

1. Markwardt, F., *Methods in Enzymology*, **19**, 924-932 (1970).
2. 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).
3. Stone, S.R., and Maraganore, J.M., *Methods in Enzymology*, **223**, 312 (1993)
4. Bagdy, D., et al., *Methods in Enzymology*, **45**, 669-678 (1976).
5. Loison, G., et al., *Bio/Technology*, **6**, 72 (1988).
6. Merck Index, 11th ed., #4638 (1989).

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