

## Product Information

## Hyaluronidase from bovine testes

Type IV-S, lyophilized powder (essentially salt-free), 750-3,000 units/mg solid

**H3884**

### Product Description

CAS Registry Number: 37326-33-3

Enzyme Commission (EC) Number: 3.2.1.35

Molecular Mass: 60 kDa<sup>1</sup>

Extinction coefficient:  $E^{1\%} = 8.0$  (280 nm)<sup>2</sup>

Synonym: Hyaluronoglucosaminidase, Hyaluronate 4-glycanohydrolase

Hyaluronidase from bovine testes is a tetramer of 4 equal subunits with a molecular mass of 14 kDa each.<sup>3</sup> This glycoprotein enzyme contains 5% mannose and 2.2% glucosamine.<sup>1</sup> It randomly hydrolyzes 1,4-linkages between *N*-acetyl- $\beta$ -D-glucosamine and D-glucuronate residues in hyaluronate. It also hydrolyzes 1,4- $\beta$ -D-glycosidic linkages between *N*-acetyl-galactosamine or *N*-acetyl-galactosamine sulfate and glucuronic acid in chondroitin, chondroitin 4- and 6-sulfates, and dermatan.<sup>4</sup> The enzyme's pH optimum is 4.5 to 6.0.<sup>5</sup>

The following compounds inhibit hyaluronidase:<sup>6</sup>

- Fe<sup>2+</sup> compounds
- Fe<sup>3+</sup> compounds
- Zn<sup>2+</sup> compounds
- Cu<sup>2+</sup> compounds
- heparin
- aurintricarboxylic acid
- sulfated, nitrated, or acetylated hyaluronic acids
- sulfated cellulose esters
- sulfated chitin esters
- sulfated carboxycellulose
- sulfated xylan
- bile salts
- sulfated steroids
- hexylresorcinol
- *o*-quinones and *p*-quinones
- sulfated aliphatic alcohols

Hyaluronidase is often used in conjunction with collagenase to dissociate the extracellular matrix between cells of animal tissue, to release viable cells for use in tissue culture. Hyaluronidase may also be used to clarify synovial fluids, to make cell counts possible.

Several theses<sup>8,9</sup> and dissertations<sup>10-18</sup> have cited use of product H3884 in their protocols.

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

This enzyme is soluble in 20 mM sodium phosphate buffer (pH 7.0), with 77 mM sodium chloride and 0.1 mg/mL BSA at 1 mg/mL, yielding a clear solution.

### Storage/Stability

It is recommended that fresh solutions be prepared before use. We have not performed stability testing of frozen solutions of this product in our laboratories. One published reference cites storage of 1.0 mg/mL stock solutions of hyaluronidase in PBS at -20 °C.<sup>7</sup>

### Procedure

#### Sample synovial fluid clarification protocol

1. Prepare a 0.5 mg/mL enzyme solution in cold 20 mM sodium phosphate buffer, pH 7.0, with 77 mM NaCl and 0.1 mg/mL BSA.
2. Using a dropper, add 1 drop per mL of fluid to be treated.
3. Incubate 5 minutes at room temperature.

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