

Hyaluronidase from sheep testes

H1492276

Storage: Store in a tightly closed container at a temperature of 2°C to 8°C. If the substance is sterile, the container should also be sterile and tamper-proof.

Introduction:

Enzymes extracted from mammalian testes (e.g., sheep testes) can hydrolyze mucopolysaccharides of the hyaluronic acid type. They may contain a suitable stabilizer. Potency: Minimum 3000 IU of hyaluronidase activity per milligram (of dry substance).

Production:

Animals used for producing hyaluronidase must meet the health requirements for animals intended for human consumption.

Characteristics:

- Appearance: White or yellowish-white, amorphous powder.
- Solubility: Soluble in water, almost insoluble in acetone and absolute ethanol.

Identification:

A solution containing 100 IU of hyaluronidase in 1 mL of 9 g/L sodium chloride solution depolymerizes a 10 g/L sodium hyaluronate BRP solution at 20°C, resulting in a significant decrease in viscosity. Heating the hyaluronidase at 100°C for 30 minutes destroys this effect.

Tests:

- Appearance of Solution: The solution should be clear. Dissolve 0.10 g in water and dilute to 10 mL with the same solvent.
- pH: 4.5 to 7.5. Dissolve 30 mg in carbon dioxide-free water and dilute to 10 mL with the same solvent.
- Loss on Drying: Maximum 5.0%. Determine by drying 0.500 g at 60°C under a pressure not exceeding 670 Pa for 2 hours.
- Bacterial Endotoxins: ≤ 0.2 EU/IU.

Assay:

The activity of hyaluronidase is determined using a slope-ratio assay, by comparing the rate at which it hydrolyzes sodium hyaluronate BRP with the rate obtained using the International Standard or a reference preparation calibrated in International Units.

Substrate Solution:

In a 25 mL conical flask, add 0.10 g of sodium hyaluronate BRP, then slowly add 20.0 mL of water at 4°C. The addition rate must be slow enough to allow the substrate particles to swell (approximately 5 minutes). Maintain at 4°C and stir for at least 12 hours. Store at 4°C and use

within 4 days.

For both the test solution and the reference solution, prepare the solutions and perform dilutions at 0°C to 4°C.

- Test Solution: Dissolve an appropriate amount of the substance in hyaluronidase diluent to obtain a solution containing 0.6 ± 0.3 IU of hyaluronidase per mL.
- Reference Solution: Dissolve an appropriate amount of hyaluronidase BRP in hyaluronidase diluent to obtain a solution containing 0.6 IU of hyaluronidase per mL.

In a reaction vessel, mix 1.50 mL of phosphate buffer solution (pH 6.4) and 1.0 mL of the substrate solution, and equilibrate at $37 \pm 0.1^\circ\text{C}$. At time $t_0 = 0$ (using the first timer), add 0.50 mL of the test solution containing E milligrams of the enzyme to be tested, mix well. Maintain the mixture at $37 \pm 0.1^\circ\text{C}$ using a suitable viscometer, record the flow time t using a second timer (with 0.1-second intervals), and perform multiple measurements over approximately 20 minutes (monitoring with the first timer). Use the following viscometer: microviscometer (DIN 51 562, Part 2), capillary type MII, with a viscometer constant of approximately $0.1 \text{ mm}^2/\text{s}^2$.

$$\eta_r = \frac{k \times t_2}{0.6915}$$

Repeat the above procedure using 0.50 mL of the reference solution containing hyaluronidase BRP. Calculate the viscosity ratio using the following expression:

- K = Viscometer constant (in mm^2/s^2 , indicated on the viscometer);
- t_2 = Flow time of the solution (in seconds);
- 0.6915 = Kinematic viscosity of the buffer solution at 37°C (in mm^2/s).

Since the enzymatic reaction continues during the flow time measurement, the actual reaction time is equal to $t_0 + t/2$ (i.e., half of the flow time ($t/2$) is added to the initial measurement time t_0). Plot $(\ln \eta)^{-1}$ as a function of the reaction time ($t_0 + t/2$) (in seconds); a linear relationship should be obtained. Calculate the slope (b) of the substance to be tested and the slope (b_r) of the reference preparation. Determine the specific activity in International Units per milligram using the following expression:

- A = Specific activity of hyaluronidase BRP (in International Units per milligram).

$$\frac{b_t}{b_r} \times \frac{E_r}{E_t} \times A$$

Perform at least three complete sets of the procedure and calculate the average activity of the substance to be tested.