Hyaluronidase from sheep testes

H754922

Store at 2-8℃ long term (24 months)

Description:

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 1000 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:

1. Appearance: White or yellowish-white, amorphous powder.
2. 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:

1. Appearance of Solution: The solution should be clear. Dissolve 0.10 g in water and dilute to 10 mL with the same solvent.
2. pH: 4.5 to 7.5. Dissolve 30 mg in carbon dioxide-free water and dilute to 10 mL with the same solvent.
3. 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.
4. 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 ± 0.1°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 ± 0.1°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 mm²/s².

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

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

- K = Viscometer constant (in mm²/s², 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 mm²/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 （In r）-I 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:

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

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

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

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.