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# Hyaluronidase for Injection

» Hyaluronidase for Injection is a sterile, dry, soluble enzyme product prepared from mammalian testes and capable of hydrolyzing mucopolysaccharides of the type of hyaluronic acid. Its potency, in USP Hyaluronidase Units, is not less than the labeled potency. Hyaluronidase for Injection contains not more than 0.25 µg of tyrosine for each USP Hyaluronidase Unit. It may contain a suitable stabilizer.

**Packaging and storage**—Preserve in *Containers for Sterile Solids* as described under *Injections and Implanted Drug Products* ⟨1⟩, preferably of Type I or Type III glass, and store at controlled room temperature.

**USP Reference standards** ⟨11⟩—

USP Hyaluronidase RS
USP L-Tyrosine RS

**Sterility Tests** ⟨71⟩ **:** meets the requirements.

**Bacterial Endotoxins Test** ⟨85⟩ —It contains not more than 2.30 USP Endotoxin Units per USP Hyaluronidase Unit.

**Limit of tyrosine**—Dissolve the entire contents of 1 or more containers of Hyaluronidase for Injection in sufficient water, accurately measured, to give a concentration of about 60 USP Hyaluronidase Units per mL. Transfer 2.0 mL of the solution to a 15-mL centrifuge tube calibrated at 6 mL, and evaporate at 105° to dryness. Add 200 µL of 6 N sodium hydroxide, and heat with steam under pressure at 121° for 3 hours. Add 300 µL of 7 N sulfuric acid, then add 1.5 mL of water and 1.5 mL of a 15 in 100 solution of mercuric sulfate in 5 N sulfuric acid. Heat on a steam bath for 10 minutes, and cool to room temperature. Add 1 mL of 7 N sulfuric acid and 1 mL of sodium nitrite solution (1 in 500), with shaking. Add water to make 6 mL, mix, centrifuge, and decant the supernatant. Twenty minutes after diluting to 6 mL, determine the absorbance of the supernatant at 540 nm, with a suitable spectrophotometer.

Repeat the preceding test, using the same quantities of the same reagents and in the same manner but omitting the Hyaluronidase for Injection and replacing the 1.5 mL of water with 1.5 mL of a solution of USP L-Tyrosine RS in 0.4 N sulfuric acid containing 30 µg in each mL.

Calculate the quantity, in µg, of tyrosine in the 2-mL aliquot of the solution of Hyaluronidase for Injection taken by the formula:

$$45(A_U/A_S)$$

in which $A_U$ and $A_S$ are the absorbances of the solution from Hyaluronidase for Injection and the Standard solution, respectively: not more than 0.25 µg of tyrosine is found for each USP Hyaluronidase Unit.

**Assay**—

*Acetate buffer solution*—Dissolve 14 g of potassium acetate and 20.5 mL of glacial acetic acid in water to make 1000 mL.

*Phosphate buffer solution*—Dissolve 2.5 g of monobasic sodium phosphate, 1.0 g of anhydrous dibasic sodium phosphate, and 8.2 g of sodium chloride in water to make 1000 mL.

*Hydrolyzed gelatin*—Dissolve 50 g of bacteriological gelatin in 1000 mL of water, heat in an autoclave at 121° for 90 minutes, and freeze-dry the solution.

*Diluent for hyaluronidase solutions*—Mix 250 mL of *Phosphate buffer solution* with 250 mL of water and, within 2 hours before use, dissolve 330 mg of *Hydrolyzed gelatin* in the mixture.

*Serum stock solution*—Constitute dried horse serum with water to its original volume, and dilute it with 9 volumes of *Acetate buffer solution.* Adjust with 4 N hydrochloric acid to a pH of 3.1, and allow the solution to stand at room temperature for 18 to 24 hours. Store the solution at 0° to 4°, and use within 30 days.

*Serum solution*—On the day of the assay, dilute 1 volume of *Serum stock solution* with 3 volumes of *Acetate buffer solution,* and adjust to room temperature.

*Potassium hyaluronate stock solution*—Prepare a stock solution to contain, in each mL, 500 µg of potassium hyaluronate, previously dried in vacuum over phosphorus pentoxide for 48 hours. Do not keep the hyaluronate over phosphorus pentoxide indefinitely. The use of a weighing bottle is desirable. Store the solution at a temperature not exceeding 5°, and use within 30 days.

*Hyaluronate solution*—On the day of the assay, dilute 1 volume of *Potassium hyaluronate stock solution* with 1 volume of *Phosphate buffer solution.*

*Standard solution*—Dissolve a suitable quantity of USP Hyaluronidase RS, accurately weighed, in cold *Diluent for hyaluronidase solutions* to obtain a solution having a known concentration of about 1.5 USP Hyaluronidase Units in each mL. Prepare this solution immediately before

use in the assay.

*Assay solution*—Dissolve the contents of 1 container of Hyaluronidase for Injection by adding cold *Diluent for hyaluronidase* directly to the container. On the basis of trial or experience, dilute the solution so prepared with cold *Diluent for hyaluronidase* so that the observed absorbances with the three dilutions of Hyaluronidase for Injection fall on the upper, linear part of the standard curve prepared as directed below.

*Procedure*—Prepare a standard concentration-response curve by adding to each of twelve 16- × 100-mm test tubes 500 µL of *Hyaluronate solution.* To each of two of the tubes add, respectively, 500, 400, 300, 200, 100, and 0 µL of *Diluent for hyaluronidase solutions.* If quantities of *Standard solution* other than those indicated below are used, change the above-specified quantities of *Diluent for hyaluronidase solutions* so that the final volume of solution in each tube, after the addition of the *Standard solution,* is 1.00 mL.

At 30-second intervals, accurately timed, add to each of two tubes 0, 100, 200, 300, 400, and 500 µL, respectively, of *Standard solution.* Mix the contents by gentle shaking, and place each tube in a water bath maintained at 37 ± 0.2°. After 30 ± 0.25 minutes, remove each tube, in order, from the water bath at 30-second intervals, and immediately add 4.0 mL of *Serum solution.* Shake the tube, and allow to stand at room temperature for 30 ± 2 minutes. Again shake the tube, and determine the absorbance at 640 nm, with a suitable spectrophotometer. Perform a blank determination but omit the hyaluronate, and make any necessary correction. Plot the average absorbance value for each level against the hyaluronidase activity expressed in Units, and draw the smooth curve that best fits the plotted points.

Concurrently, to six test tubes add 500 µL of *Hyaluronate solution* and sufficient *Diluent for hyaluronidase solutions* so that the final volume, after the addition of the *Assay solution,* is 1.00 mL. Add, at 30-second intervals, sufficient *Assay solution* so that duplicate tubes contain about 0.30, 0.50, and 0.70 Unit, respectively. Shake each tube gently, and treat as directed in the preceding paragraph for the *Standard solution,* beginning with “place each tube in a water bath.” Measure the absorbances in the spectrophotometer, and make any necessary correction for the blank. The potency of Hyaluronidase for Injection is the average of the six activity values read from the standard curve.

**Auxiliary Information** - Please check for your question in the FAQs before contacting USP.

| Topic/Question | Contact | Expert Committee |
|---|---|---|
| HYALURONIDASE FOR INJECTION | Mandy Alger<br>Scientific Liaison | BIO2 Biologics Monographs 2 - Proteins |

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