Catalog Number: 100740, 151276, 151277

Hyaluronidase

CAS # : 37326-33-3

E.C. 3.2.1.3.5

Description: Hyaluronidase catalyzes the random hydrolysis of 1,4-linkages between 2-acetamido- 2-deoxy- b-D-glucose and D-glucose residues in hyaluronate.

Source: *Bovine testes*

Solubility: Readily soluble in sodium phosphate (5 mg/ml), 0.45% sodium chloride, NaOH, 0.01% bsa pH 6.9 to give a clear, pale brown solution. Soluble in distilled water or dilute buffer.

Molecular weight: Borders and Raftery (1968) reported a molecular weight of 61,000. Khorlin, et. al. (1973) report four subunits of 14,000 each and a total molecular weight of 55,000.

Optimum pH: 4.5 - 6 (DeSalequi et al. 1967).

Composition: The enzyme is a glycoprotein containing 5% mannose and 2.17% glucosamine. The amino acid composition has been determined (Borders and Raftery 1968).

Extinction coefficient: = approximately 8.

Inhibitors: Fe^{2+} and Fe^{3+} are inhibitory as are Mn^{2+} and Cu^{2+} (Warren et al. 1962).

Specificity: Testicular hyaluronidase hydrolyzes the endo-N-acetylhexosaminic bonds of hyaluronic acid and chondroitin sulfuric acids A and C (but not B), primarily to tetrasaccharide residues (Ludowieg et al. 1961). Monosaccharides are not liberated (Rappaport et al. 1951).

Stabilizers: Yang and Srivastav (1975) report that sodium chloride acts as a stabilizer.

Assay

Method: Based on that of Tolksdorf et al. (1949) and Kass and Seastone (1944). Hyaluronic acid is measured by its ability to form turbidity with an acid albumin solution. Turbidity is a function of hyaluronic acid concentration and can hence be related to enzyme activity. One unit is the amount of enzyme that will cause the same turbidity reduction as 1.0 unit of International Standard preparation.

Reagents

- 0.1 M Sodium phosphate buffer, pH 5.3 with 0.15 M sodium chloride (HSE buffer)
- 0.5 M Sodium acetate buffer, pH 4.2
- Albumin reagent: Prepare by dissolving 2.5 grams of bovine serum albumin, Fraction V in 250 ml of 0.5 M sodium acetate buffer, pH 4.2. Adjust pH to 3.0 with 2 N HCl and heat at 93^{o}C for 30 minutes. Cool and adjust final volume to 1000 ml with 0.5 ml sodium acetate buffer, pH 4.2.
- USP/NF/International Standard: Prepare stock solutions of 1.0 and 0.5 mg/ml.
- Hyaluronic acid (HA): Dissolve 10 mg hyaluronic acid in 25 ml 0.1 M sodium phosphate buffer: pH 5.3 with 0.15 M sodium chloride. Note: This solution can be prepared by allowing the hyaluronic acid to dissolve overnight. Heating in a boiling water bath for 10 - 15 minutes is the preferred method if the material is not

immediately soluble.

Enzyme

Prepare stock solution of enzyme at one mg/ml in 0.1 M sodium phosphate buffer pH 5.3 with 0.15 M sodium chloride. Immediately prior to use dilute further in the same buffer. For crude grade material concentrations of 0.01 - 0.05 mg/ml are recommended. For purified grade concentration of 0.001 - 0.01 mg/ml are recommended.

Procedure:

I. Standard Curve

Into a series of numbered tubes, pipette as follows:

Tube #1	ml HA	mg HA	ml HSE buffer
1	0.00	0.00	1.00
2	0.10	0.04	0.90
3	0.20	0.08	0.80
4	0.25	0.10	0.75
5	0.30	0.12	0.70
6	0.40	0.16	0.60
7	0.50	0.20	0.50
8	0.60	0.24	0.40
9	0.70	0.28	0.30
10	0.80	0.32	0.20

Place all tubes in a boiling water bath for 5 minutes. Cool to room temperature. Add 9.0 ml of albumin reagent and allow to stand for 10 minutes. Read absorbance at 540 nm. Plot absorbance at 540 nm versus mg HA to form standard curve.

Hyaluronic acid should be soluble under the defined conditions and should produce a standard curve with a slope of 1.5 or greater.

II. Test Procedure

Pipette 0.5 ml of a 0.4 mg/ml hyaluronic acid solution into a series of test tubes. Incubate at 37^{o}C for 4 - 5 minutes to achieve temperature equilibrium. Incubate one blank tube with one ml of 0.1 M sodium phosphate buffer, pH 5.3 with 0.15 M sodium chloride. At timed intervals add 0.5 ml of appropriately diluted enzyme or NF standard to respective tubes. Incubate each tube exactly 10 minutes and cool in an ice bath to room temperature. Add 9.0 ml of albumin reagent to each tube and incubate at room temperature for 10 minutes. Read A540 of each tube versus the blank.

Calculation

Determine the amount of hyaluronic acid remaining after digestion from the standard curve. Calculate the amount of hyaluronic acid digested as follows:

mg HA digested = 0.2 mg - mg HA remaining

Calculate turbidity reducing units/mg of enzyme or standard as follows:

TRU/mg = (mg HA digested X 3.0) / (mg enzyme in reaction)

Calculate USP/NF units/mg enzyme as follows:

$$\frac{\text{(USP/NF/International units/mg of standard)}}{\text{(TRU /mg of standard)}} = \frac{\text{(USP/NF/International units/mg of sample)}}{\text{(TRU/mg of sample)}}$$

Availability:

Catalog Number	***Description***	***Size***
100740	Hyaluronidase, from bovine testes, activity approximately 300 USP units/mg solid	100 mg 500 mg 1 g
151276	Hyaluronidase, from bovine testes, activity approximately 2500 units/mg solid	3 KU 15 KU 30 KU
151277	Hyaluronidase, from bovine testes, activity approximately 2000 units/mg solid	100 mg 250 mg 500 mg

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