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ImmunO™

Hyaluronidase
(From Streptomyces hyalurolyticus)
Catalog #: 32042
Lot #: QR12582

Other Names: Hyaluronate lyase
Hyaluronidase, Mucinase

EC #: 4.2.2.1

Description: The hyaluronidase is purified from *Streptomyces hyalurolyticus*. This enzyme belongs to the endo-beta-hexosaminidase and endo-eliminase type enzyme that produces oligo-saccharide (unsaturated tetraose and pentose) by hydrolyzing the beta-1,4-linkage of hyaluronic acid. Hyaluronidase is stable in both high temperature and acid or alkaline range. It has high substrate specificity and hydrolyzes hyaluronic acid only in mucopolysaccharide.

Hyaluronidase
Activity: 201 TRU/ampule

Specific Activity: 6930 U/mg

Specification: Activity: More than 100 TRU/ampoule. (2,000 TRU/A_{280})
Specific Activity: More than 2000 Units/mg
Protease: For information only
Source: *Streptomyces hyalurolyticu*
Form: Lyophilized, salt free
Identity Test: Passes

Reconstitution: The volume to use is dependent on the application in which the enzyme will be used:
For digestioin of synovial fluid, reconstitute with 0.4M acetate buffer (pH 6.0) to 100 TRU/ml. Digest 100□l of synovial fluid with 10 □l of enzyme.
For immunohistochemical applications, reconstitute to 1000 TRU/ml with acetate buffer. Layer over fixed tissue sections.

Assay for
Enzyme Activity: One unit is defined as the quantity of enzyme that causes 50% of decrease in absorbance at 660 nm in 30 minutes at 60°C.
The assay described below can be used to determine the enzyme activity, which information is given above.

Method:
A modified Tolkdorf's method is applied as follows:[9]
Reaction Mixture:

1. Acetate buffer:
0.02M sodium acetate-acetic acid buffer, pH 6.0, to which NaCl is added to give a concentration of 0.15M.

2. Hyaluronic acid solution:
50 mg of purified potassium hyaluronate [as it contains 38% of glucuronic acid] from umbilical cord (Hyaluronic acid, K salt from human umbilical cords) are dissolved in 100 ml of the acetate buffer.

3. Acid bovine serum albumin solution:
Acid bovine serum albumin is measured at 280 nm and the O.D. of albumin is adjusted to 910 O.D.units/ml, weighing exactly.
The weighed albumin is dissolved to 100 ml with 0.5M acetate buffer, pH 4.3. At this time, A_{280} of this solution must be 9.1 O.D. units/ml exactly.

The pH of albumin solution is adjusted to 3.1 with 4N-hydrochloric acid and the solution isboiled in water bath for 30 minutes at 100°C and is adjusted to 100 ml with distilled water. Thereafter, it is stored at 4 - 5°C and in this form is stable for 3 to 4 weeks.

For use, the albumin solution is diluted with 5 volumes of the same acetate buffer.

4. Enzyme Solution:
Add 2.0 ml of deionized water into an ampoule of hyaluronidase to dissolve.
Pipette 1.0 ml of solution and fill up to 50.0 ml with reaction buffer (acetate buffer without sodium chloride).

Procedure:
0.5 ml of the enzyme solution (made up with the same acetate buffer without NaCl) is mixed with 0.5 ml of hyaluronic acid solution. After 30 minutes incubation at 60°C, 4 ml of acidic bovine serum albumin solution is added to the mixture. The reaction mixture is further kept for 10 minutes at the same temperature and the turbidity produced are determined at 660 nm. The readings are corrected by subtracting the measured value from the blank value, in which heat inactivated enzyme (100°C, 10 minutes) is applied instead of the enzyme solution.

Calculation:

$$\text{TRU/ml (gm)} = \frac{\text{Blank Value - Measured Value}}{\text{Blank Value}} \times 2 \times 2 \times \text{dilution}$$

Effect of Enzyme Concentration on Activity:

$$\frac{\text{Blank Value - Measured Value}}{\text{Blank Value}} \times 100 = \% \ (40\text{-}80\%)$$

Storage: Store the lyophilized powder at or below +8°C. After reconstitution, aliquot and store at -20°C.

Expiration Date: 10/30/2017

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