

**Enzymatic Assay of HYALURONATE LYASE
(EC 4.2.2.1)**

PRINCIPLE:

Hyaluronic Acid $\xrightarrow{\text{Hyaluronate Lyase}}$ Depolymerization Products + Hyaluronic Acid

CONDITIONS: T = 37°C, pH = 5.35, %T_{600nm}, Light Path = 1 cm

METHOD: Turbidimetric

REAGENTS:

- A. 300 mM Sodium Phosphate pH 5.35 at 37°C.
(Prepare 50 ml in deionized water using Sodium Phosphate, Monobasic, Sigma Prod. No. S-0751. Adjust pH to 5.35 at 37°C using 1 M NaOH.)
- B. 0.3 mg/ml Hyaluronic Acid (Substrate Solution)
(Prepare 20 ml of a 0.3 mg/ml solution using Hyaluronic Acid, Product No. H-1751 in reagent A. Heat gently and shake to dissolve.)
- C. 20 mM Sodium Phosphate pH 7.0 at 37°C with 77 mM Sodium Chloride and 0.01% (w/v) Bovine Serum Albumin (Enzyme Diluent)
(Prepare 250 ml in deionized water using Sodium Phosphate, Monobasic, Sigma Prod. No. S-0751, Sodium Chloride, Sigma Prod. No. S-9625, and Bovine Serum Albumin, Sigma Prod. No. A-6003. Adjust to pH 7.0 using 1 M NaOH.)
- D. 24 mM Sodium Acetate with 79 mM Acetic Acid 0.1% (w/v) Bovine Serum Albumin pH 3.75 at 37°C containing 1:1 HCl:H₂O (Acid Albumin Solution)
(Prepare 1 L in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, Glacial Acetic Acid, Sigma Prod. No. A-0808, Bovine Serum Albumin, Sigma Prod. No. A-6003. Adjust to pH 3.75 at 37°C using 5 N HCl.)
- E. Hyaluronate Lyase Solution
(Immediately before use prepare a solution containing 5 - 10 units per ml in cold Reagent C (Enzyme

Diluent)).

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REAGENTS: (continued)

F. NF Standard Hyaluronidase Solution
(Immediately before use prepare a solution containing
15 units NF Standard Hyaluronidase per ml in cold
Reagent C (Enzyme Diluent)).

PROCEDURE:

Pipette (in milliliters) the following reagents into
suitable tubes.

| | <u>Test₁</u> | <u>Test₂</u> | <u>Test₃</u> | <u>Blank</u> | <u>Std₁</u> | <u>Std₂</u> | <u>Std₃</u> | <u>Std₄</u> | <u>Std₅</u> |
|-----------|-------------------------|-------------------------|-------------------------|--------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Reagent B | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

Incubate at 37°C for 10 minutes. Then add at time zero:

| | | | | | | | | | |
|-----------|------|------|------|------|------|------|------|------|------|
| Reagent E | 1.00 | 0.75 | 0.50 | ---- | ---- | ---- | ---- | ---- | ---- |
| Reagent F | ---- | ---- | ---- | ---- | 1.00 | 0.80 | 0.60 | 0.40 | 0.20 |
| Reagent C | ---- | 0.25 | 0.50 | 1.00 | ---- | 0.20 | 0.40 | 0.60 | 0.80 |

Mix by inversion and incubate at 37°C for 45 minutes.
Then add:

| | | | | | | | | | |
|-----------|------|------|------|------|------|------|------|------|------|
| Reagent D | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
|-----------|------|------|------|------|------|------|------|------|------|

Immediately mix tubes by inversion and let stand at room
temperature for 10 minutes, then transfer to suitable
cuvettes. Read the percent transmittance (%T) versus
deionized water in a suitably thermostatted
spectrophotometer at 600 nm.

CALCULATION:

$$\text{units/mg enzyme} = \frac{r \% T_{\text{test}}}{(r \% T_{\text{standard}}/\text{NF units/RM})(\text{mg enzyme/RM})}$$

$$r \% T_{\text{test}} = \% T_{\text{test}} - \% T_{\text{blank}}$$

$$r \% T_{\text{standard}} = \% T_{\text{standard}} - \% T_{\text{blank}}$$

UNIT DEFINITION:

One unit is equivalent to one NF unit as determined by the
method described in USP XXII-NF XVII combined edition, p.
644 (1990).

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FINAL ASSAY CONCENTRATIONS:

In a 2 ml reaction mix, the final concentrations are 160 mM sodium phosphate, 39 mM sodium chloride, 0.005% (w/v) bovine serum albumin, 0.15 mg/ml hyaluronic acid, and 2.5 - 10 units hyaaronate lyase.

REFERENCES:

- (1955) *Meth. in Enzymol. I*, 172
- (1990) *USP XXII-NF XVII*, 644

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.