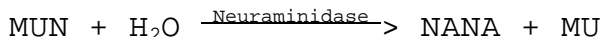


**Enzymatic Assay of NEURAMINIDASE  
(EC 3.2.1.18)**

**PRINCIPLE:**



Abbreviations used:

MUN = 2'-(4-Methylumbelliferyl)- $\alpha$ -D-N-Acetylneuraminic Acid

NANA = N-Acetylneuraminic Acid

MU = 4-Methylumbelliferone

**CONDITIONS:** T = 37°C, pH 5.5,  $A_{364\text{nm}}$ , Light path = 1 cm

**METHOD:** Stopped Spectrophotometric Rate Determination

**REAGENTS:**

- A. 1 M Sodium Acetate Buffer, pH 5.5 at 37°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 5.5 at 37°C with 1 M HCl.)
- B. 100% (v/v) N,N-Dimethylformamide  
(Use N,N-Dimethylformamide, Sigma Prod. No. D-4254.)
- C. 250 mM Glycine Buffer, pH 10.4 at 37°C (Stop Buffer)  
(Prepare 25 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 10.4 at 37°C with 1 M NaOH.)
- D. 200 mM 2'-(4-Methylumbelliferyl)- $\alpha$ -D-N-Acetylneuraminic Acid Stock Solution  
(Prepare 0.255 ml in Reagent B using 2'-(4-Methylumbelliferyl)- $\alpha$ -D-N-Acetylneuraminic Acid, Sodium Salt, Sigma Product No. M-8639. Vortex to dissolve.)
- E. 1.2 mM 2'-(4-Methylumbelliferyl)- $\alpha$ -D-N-Acetylneuraminic Acid Solution (MUN)  
(Prepare by adding 0.0125 ml of Reagent D to 0.125 ml of Reagent A. Bring to a volume of 2 ml with deionized water.)

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**REAGENTS:**

F. Neuraminidase Enzyme Solution  
(Immediately before use, prepare a solution containing approximately 0.08 unit/ml of Neuraminidase in cold Reagent A. Before use, warm the enzyme solution to 37°C.)

**PROCEDURE:**

Step 1:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent E (MUN)	0.40	0.40
Incubate at 37°C for 10 minutes. Then add:		
Reagent F (Enzyme Solution)	0.10	-----
Reagent A (Buffer)	-----	0.10

Mix by inversion and incubate at 37°C.

Step 2:

At one minute, two minute and three minute time points, remove 0.10 ml from the Test and Blank and add to predispensed aliquots of 1.00 ml of Reagent C (Stop Buffer).

Transfer the solutions to suitable cuvettes and record the  $A_{364nm}$  for both the Tests and Blank.

**CALCULATIONS:**

$$\text{Units/ml} = \frac{(A_{364nm} \text{ Test} - A_{364nm} \text{ Blank})(0.5)(1.1)(df)}{(25.3)(T)(0.1)(0.1)}$$

0.5 = Volume (in milliliter) of assay in Step 1  
1.1 = Volume (in milliliters) of stopped reaction in Step 2  
25.3 = Millimolar extinction coefficient of 4-Methylumbelliferone at 364nm  
df = Dilution factor

**Enzymatic Assay of NEURAMINIDASE  
(EC 3.2.1.18)**

**CALCULATIONS:** (continued)

T = Time (in minutes) of assay as per the Unit  
Definition

0.1 = Volume (in milliliter) of enzyme used in Step 1

0.1 = Volume (in milliliter) of Step 1 used in Step 2

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu$ mole of 2'-(4-methylumbelliferyl)  $\alpha$ -D-N-acetylneuraminic acid per minute at pH 5.5 at 37°C.

**FINAL CONCENTRATION:**

In a 0.50 ml reaction mix, the final concentrations are 250 mM sodium acetate, 0.5% (v/v) N,N-dimethylformamide, 1 mM 2'-(4-methylumbelliferyl)- $\alpha$ -D-N-acetylneuraminic acid, and 0.008 unit neuraminidase.

**REFERENCE:**

Potier, M., Mameli, L., Belisle, M., Dallaire, L., and Melancon, S.B. (1979) *Analytical Biochemistry* **94**, 287-296

**NOTES:**

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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