

**Enzymatic Assay of SIALYLTRANSFERASE
(EC 2.4.99.1)**

PRINCIPLE:

CMP-Sialic Acid + Asialomucin $\xrightarrow{\text{Sialyltransferase}}$ [¹⁴C] Mucin + CMP

Abbreviations:

CMP-Sialic Acid = Cytidine 5'-Monophosphosialic Acid

CMP = Cytidine 5'-Monophosphate

CONDITIONS: T = 37°C, pH = 6.5

METHOD: Radiometric Stopped Reaction

REAGENTS:

- A. 500 mM Sodium Cacodylate, 5% (w/v) Triton CF-54, 0.5% (w/v) Bovine Serum Albumin, pH 6.5 at 37°C (Buffer)
(Prepare 50 ml in deionized water using Cacodylic Acid, Sodium Salt, Sigma Prod. No. C-0250, Triton CF-54, Sigma Prod. No. T-0630, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust the pH to 6.5 at 37°C with either 1 N HCl or 1 N NaOH).¹
- B. 31.6 mM Bovine Submaxillary Asialomucin (Asialomucin)
(Prepare 1 ml in deionized water using Asialomucin, Sigma Prod. No. A-0789).²
- C. Cytidine 5'-Monophospho[¹⁴C]sialic acid (CMP-Sia)
(Use Cytidine 5'-Monophospho[¹⁴C]sialic Acid, Ammonium Salt, 25 µCi/ml, 150 - 310 mCi/mmol, Amersham Prod. No. CFB.165).
- D. 7.4 mM Cytidine 5'-Triphosphate (CTP)
(Prepare 1 ml in deionized water using Cytidine 5'-Triphosphate, Sodium Salt, Sigma Prod. No. C-1506).
- E. 200 mM Sodium Chloride (NaCl)
(Prepare 1 liter in deionized water using Sodium Chloride, Sigma Prod. No. S-9625).

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PROCEDURE: (continued)

- F. 4 ml Sephadex G-50 (Fine) column (Column)
(Prepare 40 ml of swelled resin in Reagent E using Sephadex G-50 Fine, Sigma Stock No. G-50-80. Pack 4 ml of swelled resin into a Column, Liquid Chromatography, 1.0 x 10 cm Luer-Lock, Sigma Prod. No. C-3794.)³
- G. Sialyltransferase Enzyme Solution (Enzyme)
(Immediately before use, prepare a solution containing approximately 0.18 unit/ml of Sialyltransferase in cold deionized water).
- H. Liquid Scintillation Cocktail (LSC)
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273).

PROCEDURES:

Prepare a reaction cocktail⁴ by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	
0.150	
Reagent B (Asialomucin)	
0.075	
Reagent C (CMP-Sia)	
0.075	
Deionized water	
0.300	

Mix thoroughly. Pipette (in milliliters) the following into suitable tubes:

	<u>Test</u>	<u>Blank</u>
Reaction cocktail	0.040	0.040

Equilibrate to 37°C, then add:

Deionized water	-----	0.010
Reagent G (Enzyme)	0.010	-----

At exactly 15 minutes, stop the reaction by adding:

Reagent D (CTP)	0.005	0.005
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Immediately place the reaction tubes on ice.⁵ Carefully transfer the contents of a reaction tube to the Sephadex G-50 column and begin elution with Reagent E (NaCl). Collect all column fractions in 7 ml scintillation vials.

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PROCEDURE: (continued)

Collect the first 1.5 ml and set aside. The initial fraction should contain only background counts. Collect the next 1.5 ml. This second fraction should be the one that contains the sialated asialomucin. Collect the next 1.5 ml fraction.³ The third fraction should contain approximately half of the remaining radiolabel as unused CMP[¹⁴C]sialic acid.

Determine the potential by pipetting 0.02 ml of the reaction cocktail in 1.5 ml of reagent E into a scintillation vial.

Add 5.5 ml of Reagent H (LSC) to each scintillation vial and immediately mix by vortexing.⁶ Count the vials for 2 to 5 minutes on a suitable scintillation counter and correct to Decays Per Minute (DPM).

CALCULATIONS:

$$\text{DPM/nmole of Potential} = \frac{(\text{DPM of potential})(0.60)}{(0.02)(\text{nmoles of CMP-Sia})(0.075)}$$

DPM = Decays Per Minute

0.60 = Volume (in milliliters) of reaction cocktail

0.02 = ml of Reaction cocktail used to determine total potential

nmoles of CMP-Sia = Total nmoles/ml of Cytidine
5'-Monophospho[¹⁴C]sialic acid in the
Reaction cocktail

0.075 = Volume (in milliliters) of CMP-Sia in the reaction cocktail

$$\text{Units/mg Enzyme} = \frac{(\text{DPM Test} - \text{DPM Blank})(0.05)(5.5)}{(\text{DPM/nmole})(15)(\text{mg enzyme/RM})}$$

0.05 = Reaction volume

5.5 = Factor⁷ to correct for inhibition due to Triton
CF-54

15 = Reaction time

RM = Reaction Mix

UNIT DEFINITION:

One unit will transfer 1.0 μmole of N-acetylneuraminic

acid from CMP-N-acetylneuraminic acid to asialomucin per minute at pH 6.5 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 0.05 ml reaction mix, the final concentrations are 100 mM sodium cacodylate, 1% (v/v) Triton CF-54, 0.1% (w/v) bovine serum albumin, 3.2 mM asialomucin, 0.008 - 0.015 mM cytidine 5'-monophospho[¹⁴C]sialic acid, and 0.002 unit sialytransferase.

REFERENCES:

Sadler, J.E., Beyer, T.A., Oppenheimer, C.L., Paulson, J.C., Prieels, J.P., Rearick, J.I., and Hill, R. (1982) in *Methods in Enzymology* (Ginsberg, V, ed.) Vol. 83, pp. 480-481

Weinstein, J., de Souza-e-Silva, U, and Paulson, J. C. (1982) *Journal of Biological Chemistry* **257**, 13835-13844

Weinstein, J., de Souza-e-Silva, U, and Paulson, J. C. (1982) *Journal of Biological Chemistry* **257**, pp 13845-13853

NOTES:

1. The Cacodylate buffer initially appears very cloudy. This is due to the solubility of the Triton. After approximately 45 minutes at room temperature with constant stirring the solution becomes clear. This solution can be stored at 0 - 5°C for several weeks.
2. The concentration is based on N-acetylgalactosamine residues, which is approximately 17 mg/ml.
3. Degas the swelled resin by aspiration prior to packing the column. The void volume of the column can be checked by using a Hemoglobin:Vitamin B₁₂ solution at 17 mg/ml Hemoglobin, Bovine, Sigma Prod. No. H-2625, and 5 mg/ml Vitamin B₁₂, Sigma Prod. No. V-2876. The Hemoglobin will elute where the Mucin should elute and the Vitamin B₁₂ will elute where any remaining CMP[¹⁴C]sialic acid should. Fraction volumes may vary and must be verified before use.
4. Keep the reaction mix on ice until ready to run the reaction.
5. Keep the stopped reactions on ice until run on the

column.

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NOTES: (continued)

6. The result will be an opaque slurry. Remove any large bubbles from the vial by gentle shaking.
7. This factor was determined empirically by kinetic analysis of the pure enzyme.
8. This assay is based on the cited references.
9. 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.