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, Triton CF-54, and Albumin, Bovine,  
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).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).

D. 7.4 mM Cytidine 5'-Triphosphate (CTP)  
(Prepare 1 ml in deionized water using Cytidine 5'-Triphosphate, Sodium Salt).

E. 200 mM Sodium Chloride (NaCl)  
(Prepare 1 liter in deionized water using Sodium Chloride).

¹ 45-1 Ramsey Road, Shirley, NY 11967, USA
Email: info@creative-enzymes.com
Tel: 1-631-562-8517 1-631-448-7888
Fax: 1-631-938-8127
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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. Pack 4 ml of swelled resin into a Column, Liquid Chromatography, 1.0 x 10 cm Luer-Lock.)

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).

**PROCEDURES:**

Prepare a reaction cocktail 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td><strong>Reaction cocktail</strong></td>
<td>0.040</td>
<td>0.040</td>
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Equilibrate to 37°C, then add:

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

At exactly 15 minutes, stop the reaction by adding:

- **Reagent D (CTP)**  
  0.005  
  0.005
Immediately place the reaction tubes on ice. 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. The third fraction should contain approximately half of the remaining radiolabel as unused CMP[$^{14}$C]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. Count the vials for 2 to 5 minutes on a suitable scintillation counter and correct to Decays Per Minute (DPM).

CALCULATIONS:

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

\[\text{DPM} = \text{Decays Per Minute}\]
\[0.60 = \text{Volume (in milliliters) of reaction cocktail}\]
\[0.02 = \text{ml of Reaction cocktail used to determine total potential}\]
\[\text{nmole of CMP-Sia} = \text{Total nmole/ml of Cytidine 5'-Monophospho[$^{14}$C]sialic acid in the Reaction cocktail}\]
\[0.075 = \text{Volume (in milliliters) of CMP-Sia in the reaction cocktail}\]

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

\[0.05 = \text{Reaction volume}\]
\[5.5 = \text{Factor}^7 \text{ to correct for inhibition due to Triton CF-54}\]
\[15 = \text{Reaction time}\]
\[\text{RM} = \text{Reaction Mix}\]

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:


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 B_{12} solution at 17 mg/ml Hemoglobin, Bovine, and 5 mg/ml Vitamin B_{12}, The Hemoglobin will elute where the Mucin should elute and the Vitamin B_{12} 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.