

Certificate of Analysis

N-Acetylneuraminic Acid Standard

| Cat. #: C | M-NEUAC-100 | Batch: B88A-01 | Expiry Date: 03 Aug 2022 | Size: ~100 nmol |
|-----------|-------------|----------------|--------------------------|-----------------|
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The N-acetylneuraminic acid standard is a quantitative standard of NIST-F and USP traceable Neu5Ac monosaccharide.



Chemical Formula: C₁₁H₁₉NO₉ Exact Mass: 309.11 Molecular Weight: 309.27 m/z: 309.11 (100.0%), 310.11 (12.5%), 311.11 (2.6%) Elemental Analysis: C, 42.72; H, 6.19; N, 4.53; O, 46.56

The bulk concentration of NeuAc was calculated independently by weight and by quantitative Nuclear Magnetic Resonance (qNMR). (Table 1) The qNMR analysis was performed in triplicate.



Figure 1. ¹H-NMR (500 MHz) of NeuAc in D_2O .



| A: Concentration by weight (mM) of NeuAc Bulk | B: Concentration using by qNMR (mM) of NeuAc Bulk | (B/A Ratio)*100 |
|---|---|-----------------|
| 28.91 | 29.15 ± 0.323 | 100.8 |

Table 1: Comparison between the concentrations calculated by weight and by qNMR of the NeuAc Bulk solution.



Figure 2. LudgerSep-R1 HPLC profile of 1,2-diamino-4,5-methylenedioxybenzene.2HCl (DMB) labelled NeuAc standard (Cat. #: CM-NEUAC-100, Batch B88A-01)

This analysis was performed on 5 vials of CM-NEUAC-100. 2 nmol from each vial were labelled. The dispensed pots were dissolved in 500 μ l of water and 10 μ l aliquots were taken for analysis. The dispensing error is predicted to be than less 5%.



DMB labeled sialic acid standards eluted under the following HPLC conditions:

Column: LudgerSep R1 (Cat. #: LS-R1-4.6x150)

<u>Flow:</u> 0.5 ml/min*.*

Temperature: 30 °C

Solvent A: methanol:acetonitrile:water (7:9:84)

Solvent B: acetonitrile

Gradient:

| Time (min) | %В |
|------------|------|
| 0.0 | 0.0 |
| 19.0 | 0.0 |
| 19.5 | 90.0 |
| 23.5 | 90.0 |
| 24.0 | 0.0 |
| 30.0 | 0.0 |
| 35.0 | 0.0 |

<u>Detector:</u> Fluorescence <u>Excitation wavelength:</u> 373 nm

Emission wavelength: 448 nm