



## **HPLC Analysis of Nucleosides, Nucliotides and Sugar Nucleotides**

### **MATERIALS**

- 50% NaOH (Fisher, SS254-1)
- Sodium Acetate Trihydrate (NaOAc) (VWR, JT3462-1)

#### **Preparation of 1L of 10 mM NaOH:**

- 520  $\mu$ L of 50% NaOH

Dissolve the 50% NaOH in Milli-Q water that has been filtered through a 0.2  $\mu$ m filter and bring the volume to 1 L. Thoroughly mix the solution.

#### **Preparation of 1L of 100 mM NaOH:**

- 5.2 mL of 50% NaOH

Dissolve the 50% NaOH in Milli-Q water that has been filtered through a 0.2  $\mu$ m filter and bring the volume to 1 L. Thoroughly mix the solution.

#### **Preparation of 1L of 10 mM NaOH with 1 M NaOAc:**

- 136.1 g of Sodium Acetate Trihydrate
- 520  $\mu$ L of 50% NaOH

Dissolve the Sodium Acetate Trihydrate in Milli-Q water that has been filtered through a 0.2  $\mu$ m filter. Then add the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution.

#### **Preparation of 1L of 100 mM NaOH with 1 M NaOAc:**

- 136.1 g of Sodium Acetate Trihydrate
- 5.2 mL of 50% NaOH

Dissolve the Sodium Acetate Trihydrate in Milli-Q water that has been filtered through a 0.2  $\mu$ m filter. Then add the 50% NaOH and bring the volume to 1 L. Thoroughly mix the solution.

### **HPLC OF NUCLEOSIDES AND NUCLEOTIDES:**

- Column:
  - Dionex CarboPac PA1 column 4 mm x 250 mm, 4 $\mu$ m, with a 4 mm x 50 mm Guard
- Solvents:
  - A: Milli-Q Water
  - B: 10 mM NaOH
  - C: 10 mM NaOH with 1 M NaOAc
  - Initial conditions of 89% A, 10% B, and 1% C at 1.0 mL/min
- UV Detector:
  - Wavelength: 260 nm
  - Data Collection Rate: 5.0 Hz



- Rise Time: 5.0 s
- Gradient Settings:

Time (min)	Water	10 mM NaOH	10 mM NaOH 1M NaOAc
0	89%	10%	1%
10	20%	30%	50%
25	15%	30%	55%
35	0%	20%	80%
40	0%	0%	100%
50	0%	0%	100%
55	0%	50%	50%
60	89%	10%	1%
80	89%	10%	1%

**HPLC OF SUGAR NUCLEOTIDES:**

- Column:
  - Dionex CarboPac PA1 column 45 µm, 4 x 250mm with 4 x 50 mm Guard
- Solvents:
  - A: Milli-Q Water
  - B: 100 mM NaOH
  - C: 100 mM NaOH with 1 M NaOAc
  - Initial conditions of 89% A, 10% B, and 0.1% C at 1.0 mL/min
- UV Detector:
  - Wavelength: 260 nm
  - Data Collection Rate: 5.0 Hz
  - Rise Time: 5.0 s
- Gradient Settings:

Time (min)	Water	100 mM NaOH	100 mM NaOH 1M NaOAc
0	88.9%	10%	0.1%
12	88.9%	10%	0.11%
15	70%	0%	30%
25	65%	0%	35%
40	60%	0%	40%
65	35%	0%	65%
80	25%	0%	75%
87	20%	0%	80%
87.5	89%	10%	0.1%
100	89%	10%	0.1%