



EFFICIENT EXTRACTION OF PYRIDINE NUCLEOTIDES FROM MOUSE MUSCLE

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CONTEXT

Measurement of pyridine nucleotides (NMN, NAD⁺, NADH, NADP, NADPH) in biological samples is vitally important given their pivotal, multifaceted roles in intermediary metabolism. We devised robust sample processing and LC/MS/MS methods to accomplish this challenging task given the inherent instability of these metabolites. Our assay precision and accuracy were enabled by the use of a Precellys Evolution homogenizer and custom-synthesized heavy isotope-labeled internal standards. We used these methods to profile the pyridine nucleotide pool in mouse gastrocnemius.

The purpose of this application note is to demonstrate that the Precellys Evolution provides for a more efficient extraction of pyridine nucleotides from mouse gastrocnemius than manual homogenization.

MATERIALS

Automated Homogenizer: Precellys® Evolution with Cryolys Cooling System

Lysing Kit: CK28_2mL tubes (Cat #: KT03961-1-002.2)

Buffer: Ice cold 0.5 M PCA for oxidized species or 50:50 0.1 M NaOH/MeOH for reduced species

Manual Homogenizer: VWR VDL 12 hand held rotary blade homogenizer

PROTOCOL

Precellys Protocol: Lyophilized mouse tissues (20 mg each) were finely powdered using CK28_2mL tubes for 3 cycles of 20 s each at 7200 rpm with a delay time of 15 s between each cycle. Aliquots of powder (5 mg) were homogenized in another set of CK28_2mL tubes in either ice cold 0.5 M PCA (500 µL) or 50:50 0.1 M NaOH/MeOH (500 µL). Tissues were homogenized at 10 °C for 6 cycles of 20 s each at 7200 rpm with a delay time of 15 s between each cycle.

Manual Homogenization: Aliquots of powder were manually homogenized on ice for 1 minute using the solvents given above.

RESULTS

Pyridine nucleotides in the homogenate supernatants were quantitated using a Dionex 3000 HPLC/ThermoScientific Quantiva triple quadrupole mass spectrometer. The tables below show the comparison between levels of pyridine nucleotides (pmol/mg dry tissue) determined in mouse gastrocnemius homogenized using manual versus Precellys Evolution homogenization.

Method	NMN (pmol/mg)	NAPD (pmol/mg)	NAD ⁺ (pmol/mg)
Manual	5.8 ± 0.7	56 ± 9	1971 ± 87
Precellys	7 ± 1	84 ± 18	1995 ± 157

Table 1. Levels of oxidized pyridine nucleotides (n = 4) obtained from manual and Precellys Evolution homogenization.

Method	NADPH (pmol/mg)	NADH (pmol/mg)
Manual	149 ± 8	1468 ± 7
Precellys	139 ± 3	1698 ± 54

Table 2. Levels of reduced pyridine nucleotides (n = 2) obtained from manual and Precellys Evolution homogenization.

CUSTOMER

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CONCLUSION

The data demonstrate that homogenization of mouse gastrocnemius using the Precellys Evolution results in similar (NMN, NAD⁺, NADPH) and higher recoveries of pyridine nucleotides (NADP, NADH) compared to manual homogenization.

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