

ANALYSIS OF ENERGY CARRIERS

Description: LC-MS/MS method for the sensitive detection and quantification of NAD/P(H) as well as other energy carriers in cells and tissues. The detection limits depend on matrix type and input quantity. NAD-related and ATP-related require different buffer and extraction conditions. Here, we provide a method that focusses on NAD-related metabolites, ATP/GTP-related compounds can be quantified with limitations. Samples are extracted and measured by a Waters I-Class Plus LC System paired with a Sciex 6500+ QTRAP. For this method we highly suggest analysing test samples to evaluate potential biases that may derive from the sampling/harvesting of cells (see note below).

Analytes are reported as μM or pmol/mio cells or pmol/mg tissue.

Container: Eppendorf Tube or equivalent.

Optimal Volume: Plasma / cell culture medium (100 μL); Tissue (25 mg)¹; Cells (1 mio).

Minimal Volume: Plasma / cell culture medium (25 μL); Tissue (10 mg)¹; Cells (0.5 mio).

Sample Collection: Please see our detailed sample collection protocols.

Quantification: Absolute (NAD-related metabolites), using a >6 point calibration curve and $r^2 > 98\%$.

Please note: For human material, note any known presence of infectious agents

List of analytes reported

(D- and L- enantiomers are not distinguished)

Compound name	Identifier	Formula	Monoisotopic mass
Adenosine diphosphate (ADP)	HMDB0001341	C10H15N5O10P2	427.03
Adenosine monophosphate (AMP)	HMDB0000045	C10H14N5O7P	347.06
Adenosine triphosphate (ATP)	HMDB0000538	C10H16N5O13P3	507.00
ADP Ribose (ADPR)	HMDB0248024	C15H23N5O14P2	559.07
Flavin adenine dinucleotide (FAD)	HMDB0001248	C27H33N9O15P2	785.16
Guanosine monophosphate (GMP)	HMDB0001397	C10H14N5O8P	363.06
Guanosine-5'-triphosphate (GTP)	HMDB0001273	C10H16N5O14P3	522.99
Nicotinamide (NAM)	HMDB0001406	C6H6N2O	122.05
Nicotinamide adenine dinucleotide (NAD)	HMDB0000902	C21H27N7O14P2	663.11
Nicotinamide adenine dinucleotide phosphate (NADP)	HMDB0000217	C21H28N7O17P3	743.08
Nicotinamide mononucleotide (NMN)	PubChem14180	C11H15N2O8P	334.22
Nicotinic acid adenine dinucleotide (NAAD)	HMDB0001179	C21H27N6O15P2	665.10

¹ Pulverized/crushed (deep-frozen) and exact weight noted

N-methylnicotinamide (MeNAM)	HMDB0003152	C7H8N2O	136.06
Reduced nicotinamide adenine dinucleotide (NADH)	HMDB0001487	C21H29N7O14P2	665.12
Reduced Nicotinamide adenine dinucleotide phosphate (NADPH)	HMDB0000221	C21H30N7O17P3	745.09

LC conditions

Column	Premier BEH Amide 100 x 2.1mm
Temperature	35° C
Mobile phase A	5 mM ammonium acetate in H2O + 0.05% (v/v) ammonium hydroxide, pH 10
Mobile phase B	Acetonitrile + 0.05% (v/v) ammonium hydroxide, pH 10
Flow	0.4 ml/min

Notes

Measurement of cell energy carriers are highly affected by the sampling procedure. Non-standardized sampling procedures, pro-longed warming and sitting times may therefore introduce strong biases that will be visible in the data.

Ideally, samples are snap frozen instantly and stored at -80C.

Long sample preparation times will affect reduced forms of metabolites.

¹ Pulverized/crushed (deep-frozen) and exact weight noted