Nucleoside & Nucleotide Analysis by HPLC

ATP/ADP ratio, NAD pool… or whole metabolic profiles

IMPORTANT: Client-specified alterations can be accommodated.

Aims: To analyze the Nucleoside & Nucleotide content in biological samples
From a one-compound analysis (*e.g.* **ADP, ATP, NAD, NADP**…) to a whole spectrum of nucleosides and nucleotides.

A wide range of samples can be analyzed: cultured cells, blood cells, body fluids...

Do not hesitate to **contact us** for any matter of feasibility!

This service is intended for many purposes, including the analysis of nucleotides in cultured cells for which it has been specially developed to study the drug impact on the cell metabolism of nucleosides and nucleotides in comparison with untreated cells.
In this case:

- it enables to:
  - reveal the metabolic changes due to the drug action, either on the purine or on the pyrimidine pathway,
  - identify the metabolites whose levels are modified by the drug treatment,
  - trace back to the metabolic step(s) altered by the drug, and to its likely target(s).
- cell culture and treatment procedures are the followings: The choice of the cell line and culture conditions has been optimized to get highly reproducible results. Assays are usually done with human hepatoma cell line Huh7. Cells are grown in DMEM supplemented with FCS (5%), glutamine (1mM), sodium pyruvate (1mM) and maintained in exponential phase. Cells are seeded on 10cm-dishes and allowed to adhere overnight. The drug is added next day at the agreed concentration and at a cell confluence of about 50%.

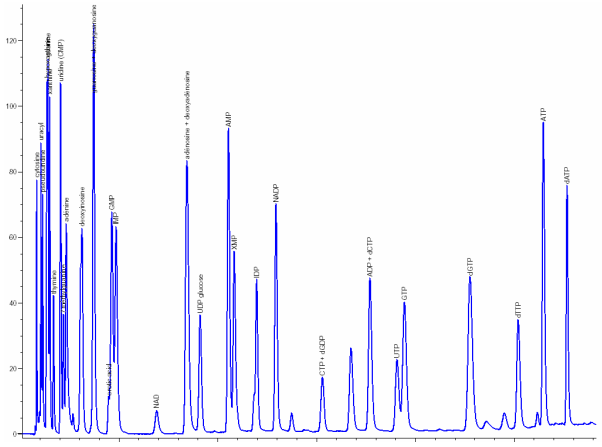
The following metabolites are routinely analyzed:

ATP	ADP	AMP	NAD	NADP
GTP	GDP	GMP	IMP	
UTP	UDP	UDP-glucose	UMP	CTP
NADH	NADPH	dATP	dGTP	dTTP
Guanine	Uracyl	Hypoxanthine	Inosine	Uridine
Cytosine	Cytidine			

Some of them, depending on the sample, are naturally present at trace level (*e.g.* IMP, hypoxanthine). However, they can be detected and quantified in appropriate larger samples or when their accumulation is due to the drug action (*e.g.* under treatment by mycophenolic acid, an IMPDH inhibitor, as shown below).

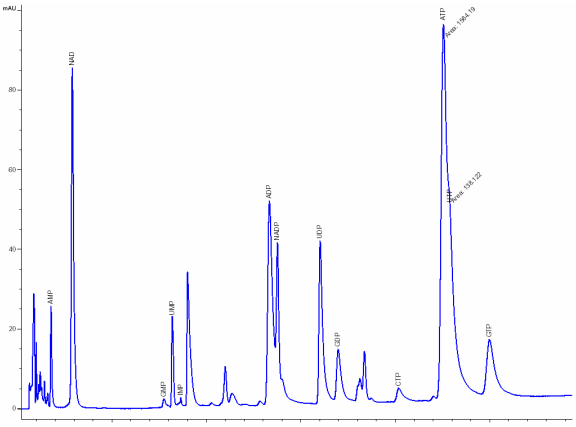
Sample size:
Cultured cell: Nucleosides & Nucleotides Analysis is usually performed by extraction of ~10^7 treated cells, per compound and per concentration tested. Control untreated cells are cultured under the same conditions to provide a reference metabolic profile.
Depending on the cell line or the experimental conditions, a 0.5-1.10^5 cell-extract can be sufficient to analyze the major metabolites (*e.g.* ATP, ADP…).
Blood cells: typically, a 200µl-sample of blood is sufficient to analyze the major metabolites in Red Blood Cells (RBC), and a 1ml-sample for Peripheral Blood Mononuclear cells(PBMC)
If needed, for instance to focus on naturally low-level metabolites, larger samples can be prepared, *e.g.* up to 10^8 of cultured cells

Nucleosides & nucleotides separation and analysis: The extraction and separation procedures have been optimized and specially developed by **NOVOCIB**. After a 48h-treatment, nucleosides and nucleotides are extracted; Nucleosides, nucleotides mono-, di-, and triphosphates, deoxynucleotides triphosphates and bases are separated by ion-pairing HPLC (Agilent 1100) with a Zorbax EclipsePlus C18 column and quantified using an Agilent ChemStation software. The resulting values are normalized by cell number. A mixture of 30 authentic standards (Sigma Aldrich, Roth) is run before and after every set of samples analysis.



Separation by ion-pairing HPLC of a 6.6pmol / 20µl standard mixture of 30 nucleosides, nucleotide mono-, di-, and triphosphates, deoxynucleotide triphosphates and bases

Quantification of intracellular metabolites in Huh-7 non-treated cells

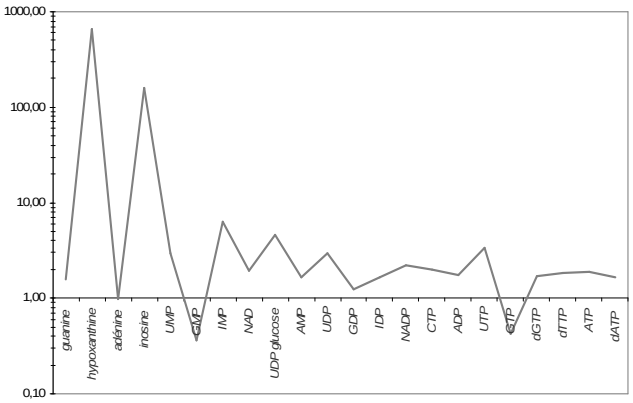


Metabolites	pmol / 10^6 cells *	published data **
UMP	728.1 +/- 56,8	
GMP	34.4 +/- 4,8	
IMP	72.7 +/- 21,4	(130)
NAD	1 825.1 +/- 155,5	
UDPglu	1 113.9 +/- 87,1	
AMP	122.7 +/- 12,9	
UDP	1 730.3 +/- 207,1	
CTP	2 754.5 +/- 165,4	
ADP	4 652.4 +/- 609,8	
UTP	2 494.2 +/- 156,1	
GTP	1 449.7 +/- 81,4	(1540)
ATP	6 561.0 +/- 346,0	(6580)

* Mean +/- SD for 5 independent experiments

** J. Balzarini *et al.* (1993): **Eicar (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide): a novel potent inhibitor of inosinate dehydrogenase activity and guanylate biosynthesis** *J. Biol. Chem.* 268 (33), 24591–24598

Metabolic signatures of mycophenolic acid, an IMPDH (inosine monophosphate dehydrogenase) inhibitor (left) and of leflunomide, a DHODH (dehydroorotate dehydrogenase) inhibitor (right) obtained in Huh-7 cultured cells.
For every metabolite, drug-treated / non-treated cells concentration ratios are calculated and graphically reported. The base line indicates the control level.



Metabolism and activation of nucleoside analogues: The separation and analytical procedures developed by **NOVOCIB** are particularly relevant to study the cellular metabolism of nucleoside analogues. The phosphorylation step by cellular kinases is crucial for the activation of nucleoside analogues and their efficacy.
At **NOVOCIB**, we routinely measure ribavirine and ribavirine mono-, di-, and tri-phosphate in cell culture extract, serum and blood cells. Quantification of other mono-, di- and triphosphorylated nucleoside analogues can be performed (subject to feasibility).
Please, see the *Cellular Pharmacology of Nucleoside Analogues* information sheet.

IP Rights: Only drugs available on the market are used to build up our metabolic profiles database. Every result obtained from the metabolic profile of any compound supplied by our client will remain its full ownership. Every related piece of information will be considered confidential and will not be transmitted to any other party.