

Gly/TCA/nucleotide (Central Metabolism Profile)

Service Code: GTN

Summary: Profile of Central Metabolism, including glycolysis, pentose-phosphate shunt, TCA cycle and nucleotide pools. One step liquid-liquid organic solvent extraction of cultured cells or tissues, separated on a 1mm x150mm HILIC specific column in a 35 min cycle. All analytes and Internal Standards are measured by ESI- ionization on a LC-QTOF mass spectrometer and reported as μM normalized to wet tissue weight, volume or cell proteins. CV's are generally 15%.

Container: 1.5mL Micro Tube or equivalent

Normal Volume: Plasma (100 μL) Tissue (50-100 mg); Cell (1.5E7).

Minimal Volume: Plasma (50 μL) Tissue (30 mg); Cells (~2.5E6)

Sample Collection: Use Snap freeze methods then store at -80°C prior to shipment for analysis is highly desired for non-plasma or cell matrix. Provide both sample weight (post freezing) and tared vial weight on sample submission.

Reference: Matthew A. Lorenz, Charles F. Burant, and Robert T. Kennedy (2011) "Reducing Time and Increasing Sensitivity in Sample Preparation for Adherent Mammalian Cell Metabolomics", *Anal. Chem.* 83(9): 3406–3414.

Table I: Analytes reported. Others on special request:

*Metabolites are low concentrations and below detection limit in some samples

Analyte	Abbr.	N(b)	Normal Conc (5x dilution)	Mol Formula	Rt	LOQ(μM)
Acetyl-CoA	aCoA	N/A	N/A	$\text{C}_{23}\text{H}_{38}\text{N}_7\text{O}_{17}\text{P}_3\text{S}$	20.70	0.2
Citrate/Isocitrate combined	Cit/i-Cit	114.2 +/- 27 μM	22.8	$\text{C}_6\text{H}_8\text{O}_7$	19.00	0.2
Succinate	Suc	8.8 +/- 2.7 μM	1.7	$\text{C}_4\text{H}_6\text{O}_4$	16.36	0.2
Malate	Mal	N/A	N/A	$\text{C}_4\text{H}_6\text{O}_5$	16.50	0.2
2-Phosphoglycerate/3-Phosphoglycerate combined	2PG/3PG	1.6 +/- 2.4 μM (infants)	.32	$\text{C}_3\text{H}_7\text{O}_7\text{P}$	19.30	0.2
Phosphoenolpyruvate	PEP	17 +/- 3.8 μM	3.4	$\text{C}_3\text{H}_5\text{O}_6\text{P}$	19.80	0.2
Adenosine monophosphate	AMP	6.2 +/- 3.1 μM	1.24	$\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_7\text{P}$	17.20	0.2
Adenosine diphosphate	ADP	160.0 +/- 14.0 μM	32	$\text{C}_{15}\text{H}_{23}\text{N}_5\text{O}_{14}\text{P}_2$	20.00	0.2
Adenosine triphosphate	ATP	1552.0 +/- 161.0 μM	310	$\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$	22.30	0.2
Flavin adenine dinucleotide	FAD	0.075 (0.056-0.097) μM	.015	$\text{C}_{27}\text{H}_{33}\text{N}_9\text{O}_{15}\text{P}_2$	16.61	0.2
Nicotinamide adenine dinucleotide	NAD	24.00 (23.00-25.6) μM	4.8	$\text{C}_{21}\text{H}_{28}\text{N}_7\text{O}_{14}\text{P}_2$	13.80	0.2
Nicotinamide adenine dinucleotide, reduced	NADH	22.0 (14.0-40.0) μM	4.4	$\text{C}_{21}\text{H}_{29}\text{N}_7\text{O}_{14}\text{P}_2$	16.50	0.2
Nicotinamide adenine dinucleotide phosphate	NADP	N/A	N/A	$\text{C}_{21}\text{H}_{29}\text{N}_7\text{O}_{17}\text{P}_3$	19.00	0.2

Nicotinamide adenine dinucleotide phosphate, reduced	NADPH	51	12.75	C ₂₁ H ₃₀ N ₇ O ₁₇ P ₃	21.00	0.2
Erythrose 4-phosphate*	E4P	N/A	N/A	C ₄ H ₉ O ₇ P	16.2	0.2
Ribulose 5-phosphate/Xylulose 5-phosphate/ribose-5-phosphate combined*	R5P/X5P/Ru5P	1.58+/- 1.31	.316	C ₅ H ₁₁ O ₈ P	15.9	0.2
6-phosphogluconate*	6PG	N/A	N/A	C ₆ H ₁₃ O ₁₀ P	18.80	0.2
Sedoheptulose 7-phosphate*	S7P	.89 +/-0.41	.178	C ₇ H ₁₅ O ₁₀ P	16.1	0.2
Fructose-6-phosphate + glucose-6-phosphate	F6P/G6P	10.2 +/- 1.8	2.04	C ₆ H ₁₃ O ₉ P	16.81	0.2
Fructose-bisphosphate	FBP	1.2 +/- 0.4	0.24	C ₆ H ₁₄ O ₁₂ P ₂	20.50	0.2

Table II: Internal standards

Internal Standards	Source	Cat#	Metabolites	Rt
¹³ C ₂ -fumarate	sigma	606073	NAD, Suc, FAD, Sed	16.36
¹³ C ₆ -citrate	sigma	606081	Hexose-6-Phosphate, NADP, 6PG, G3P	19.00
¹³ C ₆ -Fructose-bisphosphate	omicron biochem,	fru-028	FBP, NADPH, PEP, 2PG/3PG,	20.5
¹³ C ₁₀ , ¹⁵ N ₅ -ATP	sigma	645702-10MG	ATP, a-CoA, ADP	22.3
¹³ C ₁₀ , ¹⁵ N ₅ -AMP	sigma	650676	AMP, E4P, X5P/R5P, S7P,	17.200
¹³ C ₄ -L-Malic acid	sigma	750484	Mal, NADH	16.5
¹³ C ₆ -Fructose-6-phosphate			Hexose-6-Phosphate	16.80

Materials

1. Agilent 6520 QTOF with 1260 LC unit, chilled autosampler.
2. Probe or bead mechanical formatted homogenizer with appropriate beads and protocol for tissues to be analyzed
3. Vortexer
4. Refrigerated centrifuge, capable of 15,000g with microtube tube compatible rotor
5. Vacufuge with microtube compatible rotor
6. Wet and dry ice
7. 0.1 mg capable -balance
8. Prepared internal standard and authentic standards mix solutions and known concentrations.
9. Micro tubes (polypropylene), glass autosampler vials- caps
10. LCMS grade water, acetonitrile, methanol, chloroform, ammonium acetate, ammonium hydroxide
11. Appropriate instruments for liquid handling (pipettes)

Procedure:

Tissue Sample Preparation

1. Weigh frozen tissue samples and transfer to clean pre-labeled microtubes, record weight.
Homogenize tissues using cooled preferred method beaded mechanical homogenizer or probe

sonicator. Place high importance on keeping samples cool while homogenizing. Samples should be kept on dry ice till post homogenization step has occurred.

2. Add appropriate amount of extraction solution to all tubes, then vortex to mix.
3. Incubate 10 minutes on ice or placed in 2-8°C, repeat vortex..
4. Centrifuge 1 at 15,000g for 10 minutes in 4 °C.
5. Transfer supernatant into a clean, pre- labeled autosampler vial for LC-MS analysis
6. Reserve remaining tissue sample/extract at -20 or -80°C until analysis is complete.
7. Once analysis is complete, for non-plasma matrix dry extracted tissue, record weight for normalization. For cellular matrices measure extracted protein content using the preferred protein determination method.

Cell Sample Preparation

1. Put samples and extraction solvent containing appropriate internal standards on dry ice.
2. Working one plate at a time, remove plate from the cooler and place on a surface of wet ice.
3. Add 1.5 mL of extraction solvent (.750 mL twice) to the plate.
4. Scrape cells with cell scraper from culture plate bottom, collect solvent and extracted material to one side of the plate.
5. Transfer solvent and extracted material to a clean pre- labeled 1.5 -2mL microtube.. Place extracted sample on wet ice till all other samples are processed.
6. Repeat procedure with all samples.
7. Centrifuge all microtubes at 15,000g for 10 minutes in 4 °C
8. Transfer 100 µL of supernatant to clean autosampler vials (with insert). Remaining sample returned to -20 or -80 °C storage.

LC-MS procedure

1. LC column: Phenomenex Luna NH2 column, 1mm x 150mm
2. Mobile phase A: 5mM ammonium acetate in water, pH 9.9 using Ammonium Hydroxide
3. Mobile phase B: LCMS-grade 100% Acetonitrile
4. Gradient: 0min, 80%B, 15min, 0%B, 20min, 0%B, 35min, 80%B, flow rate: 0.075 mL/min
5. Autosampler: 4°C, 10 µL injection (injection volume can be changed to meet matrix concentrations).
6. Agilent 6520 Q-TOF: ESI⁻, 350 °C, drying gas 10l/min; ESI: 3500V Method: **M006-1mmNH2-35min_neg.m** or equivalent
7. Collect standard curve data first, then sample data if system is suitable.

Quantification:

External calibration curve is constructed from calibration standards and it is used to calculate metabolite concentrations in biological samples. Internal standard mixture within the sample extraction solvent and individual calibration standard curve are used to normalize data set.