UNIVERSITY OF PITTSBURGH METABOLIC CORE FACILITY

Website: https://www.pediatrics.pitt.edu/research/cores-research-support/metabolic-core

SERVICE REQUEST FORM

Send completed Service Request Forms and any inquiries to:
Clint Van't Land, PhD
email: clv19@pitt.edu / clinton.vantland@chp.edu
Tel: 412-692-7652

CONTACT INFORMATION

Director: Clint Van't Land, PhD
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PROJECT CONSULTATION

Technical consultation will be required prior to the first sample submission.

SERVICE REQUESTED

- ☐ Service #1 – LC/MS/MS (mass spectrometry) < Complete Pages 1 & 2 >
- ☐ Service #2 – Seahorse extracellular flux analyzer < Complete Pages 1 & 3 >
- ☐ Service #3 – Amino Acid analyzer < Complete Pages 1 & 4 >
- ☐ Service #4 – HPLC with UV(PDA)/Fluor/ECD < Complete Pages 1 & 5 >
- ☐ Service #5 – Spectrofluorometer < Complete Pages 1 & 6 >
- ☐ Service #6 – Spectrophotometer < Complete Pages 1 & 6 >

USER INFORMATION

Principal Investigator

Name: Date:

Requestor/Contact Name:

Phone Number:

Email:

ACCOUNT NUMBER (Enter in Line 1, 2 or 3)

1. University Account # (32-digit)
2. UPMC Account # (BU & Cost Ctr 10-digit):
3. Purchase Order #:

Name of Project:

Approved IACUC protocol #:

Approved IRB protocol I#:

Biosafety Level: BSL-1 ☐ BSL-2 ☐ Infectious Agent ☐

Service #1 Sample Information Page – LC/MS/MS (mass spectrometry)

TYPE OF ANALYSIS

☐ Acylcarnitines ☐ Acyl-coenzyme A ☐ New Assay Development (Provide publication)

SAMPLE INFORMATION

Number of samples to be analyzed:

Anticipated Sample Submission Date (prior to analysis):

Sample type:

☐ Plasma ☐ Cell medium ☐ Cell pellet ☐ Animal tissue ☐ Urine ☐ Other

Sample volume (µL): (preferred container size: 1 – 2 mL tube or vial)

Sample concentrations: (indicate unit - ng/mL or µM)

- The current Limit of Detection (LOD) for the analysis of acylcarnitines is ≥ 0.1 µM (100 nM).

List all compounds to be analyzed in each sample:

Compound Name / Class of Compound:	CAS#:	MW:

Internal Standards:

List MW of any known MS/MS fragments using tandem mass spectrometry:

Method of Sample preparation (if known/required): (Please describe in detail any extraction procedures, reconstitution solutions used, etc. The use of inappropriate buffers and/or nonvolatile solutions may result in incomplete sample analysis. Avoid solvents with high boiling points (DMSO), salts, and detergents in sample preparation.)

Service #2 Sample Information Page – Seahorse Analyzer

ALL REQUESTS must be discussed with the Metabolic Core Staff to optimize the cell culture conditions and plating techniques to achieve the best cell analysis results!!

TYPE OF ANALYSIS

Type of Cells (e.g. fibroblasts, hepatocytes): _____ Human ☐ Animal ☐

Choose Assay Kit:

☐ Mito Stress ☐ Glycolysis Stress ☐ ATP Rate ☐ Glycolytic Rate

☐ Palmitate-BSA FAO ☐ Mito Fuel Flex ☐ Cell Energy Phenotype ☐ Other

Published Method / Data: Please provide (attach) any published methods or data for discussion with Staff.

_____

** NOTE: All plates will be analyzed using the "Default" Protocols unless the Requestor specifies a desire to use a Custom Analysis or Protocol (i.e. optimization of function and run time).

PLATE LAYOUT INFORMATION

Number of samples/columns to be populated: _____

** *Warning: All 96 wells need to contain a solution to prevent damage to the instrument!!* **

Anticipated Submission Date: _____

Plate Layout Template: *<Request file from Core Staff – sent via email>*

PRE-SUBMISSION CHECKLIST

Day 1 < One day prior to analysis >

☐ Arrange to pick-up Agilent Supplies from Core Staff

Supplies Include: Kit (foil pouch), Cell Culture plate, Sensor Cartridge w/ Utility plate, coating, calibrant, medium

Cell Culture Plate preparation

☐ Coat the Cell Culture plate with Poly-D-Lysine (default) solution <Note: coating is optional>

☐ Plate cells at optimized density (as previously determined) in appropriate Agilent medium and incubate overnight

Sensor Cartridge preparation per Agilent Kit User Guide

☐ Add Calibrant solution to Utility Plate & place Sensor Cartridge on top

☐ Hydrate Sensor Cartridge at 37 °C in non-CO_2 incubator overnight

Day 2 < Day of Analysis >

☐ Prepare and Load Reagent solutions into Sensor Cartridge at volumes described in Kit User Guide

Additional Services - Fee charged at Project Consultation rate – see *Metabolic Core Price List*

☐ Protein Assay for data normalization

☐ Statistical Analysis / Data Interpretation

Service #3 Sample Information Page – Amino Acid analyzer

TYPE OF ANALYSIS

☐ Amino Acids List of AAs most relevant to your project:

☐ New Assay Development (alternate sample type, e.g. cell medium or CSF)

NOTES TO REQUESTOR:

The current method(s) employs a HPLC system with a post-column reactor (Pickering Laboratories).

Method Type (1 or 2):

1. Full Panel Analysis – 42 amino acids with a run time of 152 minutes (2-1/2 hours) per sample
2. Partial List Analysis – 1 to 4 amino acids with a run time of 64 minutes (1 hour) per sample

Please discuss which Method (1 or 2) should be used for your project prior to sample submission.

SAMPLE INFORMATION

Number of samples to be analyzed:

Anticipated Sample Submission Date (prior to analysis):

Sample type: ☐ Plasma ☐ Urine

Sample volume: (preferred container size: 1 – 2 mL tube or vial)

Sample concentrations: (ng/mL or µM)

Service #4 Sample Information Page – HPLC with UV(PDA)/Fluor/ECD

TYPE OF ANALYSIS

☐ Drug(s) or metabolite(s) ☐ Flux studies ☐ Native protein tryptic mapping

Published Method / Data: Please provide any published methods or data for discussion with Staff.

SAMPLE INFORMATION

Number of samples to be analyzed:

Anticipated Sample Submission Date (prior to analysis):

Sample Extract from:

☐ Plasma ☐ Cell medium ☐ Cell pellet ☐ CSF ☐ Urine ☐ Other

Sample Extract volume (µL): (preferred container size: 1 – 2 mL tube or vial)
Or
Reconstitute Volume from Dry Residue (µL):

- If reconstitution required, provide the composition of the reconstitute solution and final volume.

Sample concentrations: (ng/mL or µM)

Limit of detection required: (ng/mL or µM)

List all compounds to be analyzed in each sample:

Compound Name / Class of Compound:	CAS#:	MW:

Method of Sample preparation (if known/required): (Please describe in detail any extraction procedures, reconstitution solutions used, etc. The use of inappropriate buffers and/or nonvolatile solutions may result in incomplete sample analysis. Avoid solvents with high boiling points (DMSO), salts, and detergents in sample preparation.)

Service #5 Use Information Page – Spectrofluorometer

TYPE OF ANALYSIS

☐ Fluorescence measurements ☐ Kinetic study

SCHEDULING INFORMATION

Requested Date of Use:

Number of hours requested for analysis:

Service #6 Use Information Page – Spectrophotometer

TYPE OF ANALYSIS

☐ Absorbance measurements ☐ Kinetic study

SCHEDULING INFORMATION

Requested Date of Use:

Number of hours requested for analysis: