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**SERVICE REQUEST FORM**

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Send completed Service Request Forms and any inquiries to:

Clint Van't Land

email: [clv19@pitt.edu](mailto:clv19@pitt.edu) / [clinton.vantland@chp.edu](mailto: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 – HPLC with ECD/UV(PDA)/FLR < [Complete Pages 1 & 4](#) >

**CUSTOMER INFORMATION**

Principal Investigator

Date:

Name & Title:

Requestor/Contact Name:

Contact Building & Room / Lab Location:

Email:

Phone Number: (      )

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

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 #:

Biosafety Level: BSL-1  BSL-2  Infectious Agent

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

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TYPE OF ANALYSIS

FIA-MS/MS (Flow injection analysis): Run Times  $\leq$  4 minutes per injection

- Acylcarnitines (54) and/or Acylglycines (3)  Amino Acids (9)  
 New Assay Development (Provide publications)

LCA-MS/MS (LC column analysis): Run Times  $>$  4 minutes per injection using a HPLC column

- Qualified assay for SAM-SAH (S-adenosylmethionine/S-adenosylhomocysteine)  
 New Assay Development (Provide publications)

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 ( $\mu$ L): (preferred container size: 1 – 2 mL tube or vial)

Sample concentrations: (indicate unit - ng/mL or  $\mu$ M)

- The current Limit of Detection (LOD) for the analysis of acylcarnitines is  $\geq$  0.1  $\mu$ M (100 nM).

List all compounds to be analyzed in each sample:

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

Internal Standards:

List molecular weights 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.)

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**Service #2 Sample Information Page – Seahorse Analyzer**


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***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

Standard XF Kit(s) supplied by Core:  Cell Mito Stress  ATP Rate

NOTE: All Other XF Kits need to be purchased or paid for by the USER

T Cell Metabolic Profiling  Glycolysis Stress  Glycolytic Rate  
 Palmitate-BSA FAO  Mito Fuel Flex  Cell Energy Phenotype  Other

Published Method / Data: Provide (attach) 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

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

Anticipated Date of First TC Plate:

Plate Layout Template: **USER INSTRUCTIONS**

1. Download **WAVE Desktop** software from Agilent to create <template.asyt> file.  
<https://www.agilent.com/en/products/cell-analysis/cell-analysis-software/data-analysis/wave-desktop-2-6>
2. Access User's (i.e. employee/personal) OneDrive folder and Upload <template.asyt> file.
3. From Seahorse workstation, Access User's OneDrive folder and Download <template.asyt> file to User-created <folder> on C:\ drive (Seahorse workstation).
4. Using Wave Controller software, Import <template.asyt> file as Protocol file.

**Day 1 < One day prior to analysis >**

Arrange to pick-up Agilent Supplies from Core Staff

User prepared Supplies include: Kit (foil pouch), Cell Culture (i.e. TC or Spheroid Microplate) plate, coating, and XF Base medium and Supplemental solution (i.e. glucose, glutamine, pyruvate)

Staff prepared Supplies include: Sensor Cartridge w/ Utility plate, and calibrant

**Cell Culture Plate preparation (by USER)**

- Coat the Cell Culture (TC) plate with Poly-D-Lysine (default) solution or another cell adhesive.  
 Plate cells at optimized density in appropriate Agilent medium and incubate overnight

**Sensor Cartridge preparation (by STAFF)**

- Add Calibrant solution to Utility Plate & place Sensor Cartridge on top  
 Hydrate Sensor Cartridge at 37 °C in non-CO<sub>2</sub> incubator overnight

**Day 2 < Day of Analysis >**

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

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**Service #3 Sample Information Page – HPLC with ECD / UV(PDA) / FLR**

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TYPE OF ANALYSIS

Drug(s) or metabolite(s)     Flux studies

Published Method / Data: Please provide (attach) 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 ( $\mu\text{L}$ ):                      (preferred container size: 1 – 2 mL tube or vial)

Or

Reconstitute Volume from Dry Residue ( $\mu\text{L}$ ):

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

Sample concentrations:                      (ng/mL or  $\mu\text{M}$ )

Limit of detection required:                      (ng/mL or  $\mu\text{M}$ )

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.)