**Acyl-CoA Profile**

**Service Code: Acyl-CoA**

**Summary:** Profile 5 long-chain acyl-CoA species by mixed organic solvent extraction. Extracts are dried, re-suspended and measured by ESI+ ionization on a LC-QQQ mass spectrometer using MRM methods. Analytes are reported as uM, with CV's generally ~10%.

**Container:** 1-2 mL polypropylene centrifuge tube

**Normal Volume:** Tissue (50-100 mgs); Cells (2E7). NB: Normally only found in tissues

**Minimal Volume:** Tissue (30 mg); Cells (~5E6)

**Special Handling:** If human or primate, note any known presence of infectious agents.

**Sample Collection:** Snap freeze by liquid nitrogen. For tissues, resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

**Reference:** Haynes, CA et al. (2008) "Quantitation of fatty-acyl-coenzyme As in mammalian cells by liquid chromatography-electrospray ionization tandem mass spectrometry" *J Lipid Res.* 49(5), 1113-1125. Haynes, CA (2011) "Analysis of mammalian fatty acyl-coenzyme A species by mass spectrometry and tandem mass spectrometry" *Biochim et Biophys Acta* 1811(11), 663-668.

**Table I: Analytes reported. Others on special request:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Analyte** | **Abbr.** | **PubCHEM** | **LOQ(uM)** |
| CoASH | CoA | 87642 |  |
| Acetyl-CoA | (2:0)-CoA | 444493 |  |
| Propionyl-CoA | (3:0)-CoA | 92753 |  |
| Butyryl-/isobutyryl-( combined)-CoA | (4:0)-CoA | 439173/3036931 |  |
| Isovaleryl-CoA | (5:0i)-CoA | 439855 |  |
| Malonyl-CoA | (mal)-CoA | 10663 |  |
| Methylmalonyl-CoA | (Mmal)-CoA | 123909 |  |
| Succinyl-CoA | (suc)-CoA | 439161 |  |
| NB: Red Font: short-chain CoA's done as separate assay |  |  |  |
|  |  |  |  |
| Palmitoyl-CoA | (16:0)-CoA | 52922017 |  |
| Palmitoleoyl-CoA | (16:1)-CoA | 25244394 |  |
| Stearoyl-CoA | (18:0)-CoA | 94140 |  |
| Oleoyl-CoA | (18:1)-CoA | 5497111 |  |
| Linoleoyl-CoA | (18:2)-CoA | 6441626 |  |
| Arachidyl-CoA | (20:0)-CoA |  |  |
| Arachidonyl-CoA | (20:4)-CoA |  |  |
| Behenyl-CoA | (22:0)-CoA |  |  |
|  | (22:6)-CoA |  |  |
| Lignoceryl-CoA | (24:0)-CoA |  |  |
|  | (24:1)-CoA |  |  |
| Cerotyl-CoA | (26:0)-CoA |  |  |
|  | (26:1)-CoA |  |  |

**Table II: Internal standards and corresponding analytes**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Internal Standards** | **Source** | **Cat#** | **Analytes quantified** | mg/ml |
| 17:0 Coenzyme A | Avanti | 870717 |  | 2 |
| 25:0 Coenzyme A | Avanti | 870729 |  | 2 |

**Materials**

1. Agilent 6410 QQQ with 1260 LC unit, chilled autosampler, with standard 54-well autosampler plate
2. Vortexer
3. Refrigerated centrifuge, capable of 13,000g with eppendorf tube compatible rotor
4. Eppendorf Vacufuge
5. ice bucket, ice
6. Prepared stock solutions of acyl CoAs standards and isotope-labelled C17 CoA (internal standard).
7. eppendorf tubes (polypropylene)
8. LCMS grade water, acetonitrile, methanol
9. ACS Reagent grade chloroform, ammonium acetate, acetic acid, ammonium hydroxide

**PROCEDURES:**

**Preparation of Extraction Solvent**

1. Solvent 1: Prepare 20 mL of 8:1:1 methanol:water:chloroform (16 mL methanol, 2 mL water, 2 mL chloroform).
2. Solvent 2: 2987 uL of 8:1:1, + 3 uL of C17 AcylCoA

**Preparation of Standards**

Acyl CoA standard (100uM STD mix): (C2:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:1, C20:2, C20:3, C20:4, [C17:0, C25:0 (IS, 1.96mg/ml)] :

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Standard name/ Concentration | Volume of methanol | Volume of chloroform | Volume of water | Volume of 100 uM STD mix | Volume of extraction solvent 2 |
| STD 0 / 0 uM | 200 | 25 | 25 | 0 | 250 |
| STD 1 / 0.1 uM | 200 | 25 | 24.5 | 0.5 | 250 |
| STD 2 / 0.5 uM | 200 | 25 | 22.5 | 2.5 | 250 |
| STD 3 / 2.5 uM | 200 | 25 | 12.5 | 12.5 | 250 |

**Plasma/Serum Sample Preparation**

1. Weigh frozen tissue samples and transfer to labeled eppendorf tube and record weight. Homogenize tissue.
2. Add appropriate amount of extraction solvent to the sample in eppendorf tube, vortex to mix.
3. Keep all samples on ice for 10 min, vertex again.
4. Centrifuge all vials at 4°C and 16,000 xg for 10 minutes. Transfer supernatant to autosampler vial and Analyze by LC-MS

**Tissue Sample Preparation**

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**Cell Sample Preparation**

1. Put samples in a box with dry ice. Put extraction solvent on dry ice.
2. Working one plate at a time, remove plate from the cooler and place on a surface of regular ice.
3. Clean cell scraper with MeOH and kimwipe.
4. Add 540ul of extraction solvent to the plate.
5. Scrape cells with cell scraper, then scrape solvent to one corner of the plate.
6. Transfer debris to a labeled 2mL eppendorf vial. Put vial on dry ice, incubate 5 minutes.
7. Repeat procedure with all additional eppendorf vials.
8. Centrifuge all vials at 15,000g for 10 minutes
9. Transfer 100ul of supernatant to an autosampler vial (with insert)
10. **LCMS Calibration**

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**LC-MS procedure**

1. LC column: Waters C18 column, 2.1mm x 50mm
2. Mobile phase A: 5mM ammonium acetate in water, pH 9.9 using NH4OH
3. Mobile phase B: 100% acetonitrile
4. Gradient: 0min, 10%B, 7min, 100%B, 11min, 100%B, 16min 10%B, flow rate: 0.23/min
5. Autosampler: 4°C, 5 uL injection
6. Agilent 6410 QQQ: ESI+, Method: **QM000** or equivalent

**Table III: MRM transitions monitored:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Analyte** | **MRM 1** | **MRM 2** | **Polarity** | **Quant or qual?** |
| C16 |  |  | + |  |
| C16:1 |  |  | + |  |
| C17 |  |  | + |  |
| C18 |  |  | + |  |
| C18:1 |  |  | + |  |
| C18:2 |  |  | + |  |
| C20:1 |  |  | + |  |
| C20:2 |  |  | + |  |
| C20:3 |  |  | + |  |
| C20:4 |  |  | + |  |
| C25 |  |  | + |  |
| C2 |  |  | + |  |
| C4 |  |  | + |  |
| C5i |  |  | + |  |
|  |  |  | + |  |