Fatty Acid Oxidation of \(^{14}\text{C}\)-Palmitate
A Method for CO\(_2\) Entrapment and Isolation of Acid Soluble Products


We've performed this assay on the following cell types:

- **Dissociated Primary Cortical Neurons**
  - Plate 10 ml of cells @ 1 million cells/ml in Nunclon T-75 flasks coated with poly D-lysine.
  - Use cultures 5-8 days after plating.

- **Differentiated 3T3-L1A Adipocytes**
  - Plate pre-adipocytes at 2x10\(^5\) cells/T25 flask (Corning or Falcon)
  - Use 7-10 post differentiation to adipocytes - there will be ~5x10\(^6\) cells/flask at treatment time.

**Solubilize Palmitate:**

- Dry 100 \(\mu\)l of [1-\(^{14}\text{C}\)]-Palmitic acid under N\(_2\) in a small scintillation vial (250 \(\mu\)Ci in 2.5 ml ethanol, 53 mCi/mmol from Moravek Biochemicals).
- Resuspend in 2 ml of \(\alpha\)-CD (\(\alpha\)-Cyclodextran) – 10 mg/ml in 10 mM Tris.
- Shake gently in 37\(^\circ\)C water bath for 30 min.
- Freeze @ -20\(^\circ\)C until use. Once it is thawed for use, should not be refrozen!

**Treatment and Incubation with [1-\(^{14}\text{C}\)]-Palmitate:**

- Remove media from flask and rinse 2X with DPBS --- Keep one flask for protein determination.
- Add 890 \(\mu\)l of HAM F-10 media
  - Treat 2 hr time point flasks with drug/compound.
    - Incubate @ 37\(^\circ\)C.
    - After 1 hr, treat the “1 hr time point” flasks.
    - After another 30 min, treat the “30 min time point” flasks and add the hot mix to all flasks.

**1X ‘hot’ mix**

- 100 \(\mu\)M [1-\(^{14}\text{C}\)]-palmitate
- 200 \(\mu\)M carnitine
- HAM-F-10 media

- Already added media, therefore add 110 \(\mu\)l of [1-\(^{14}\text{C}\)]-palmitate/carnitine mix to each flask.
- Cap with rubber stopper including the hanging well containing filter paper + 10 \(\mu\)l of 20% KOH.
- Incubate @ 37\(^\circ\)C for 30 min.
- Turn on 60\(^\circ\)C water bath.

**CO\(_2\) Entrapment:**
• Stop reaction by injecting 200 μl of 2.6N HClO₄ do not remove the rubber stopper as this will release the CO₂ before it can be ‘trapped’ by the KOH.
• Trap for 2 hrs @ 37°C with flasks standing up.
• Count filter paper using budget solve scint. fluid – add 5 ml scintillation fluid to filter paper in small vial and allow to sit for approx. 1 hr before counting.

**Isolation of Acid Soluble Products:**
- To hydrolyze the esters – transfer contents of flask to a glass tube and add 200 μl of 4 N KOH.
- Incubate in 60°C water bath for 30 min.
- Then add 300 μl of 1 M NaAc and 200 μl of 3 N H₂SO₄ - vortex to mix.
- Spin for 7 min in desk top centrifuge (1000 rpm).
- Remove 900 μl of sup. for extractions:
  - Add 3.75 mls of CHCl₃:MeOH - vortex
  - Add 1.875 ml CHCl₃ – vortex
  - Add 1.125 ml H₂O – vortex twice very well
  - Spin on setting (1000 rpm for TC room centrifuge)
  - Remove upper phase and count with Budget Solve in large scintillation vial.

**Ordering information:**
- Rubber sleeve stoppers: VWR# 59586-410; size 15.
- Hanging center wells: VWR #72760-048
- [1-¹⁴C]-Palmitic acid: Moravek Biochemicals; 250 μCi in 2.5 ml ethanol, 53 mCi/mmol.
- L-Carnitine Hydrochloride: Sigma # C-7518.