

## **C<sub>2</sub>C<sub>12</sub> Myotube Glucose Oxidation**

Kelly lab 2005

### **Glucose Oxidation**

1. Plate cells (10% FCS; DMEM (4.5 g/l))
2. Change media when > 90% confluent (2% HS; DMEM (4.5 g/l))
  - a. Wash 2 X PBS prior to change
  - b. Refresh media and infect 3-4 days later; change media every 48-60 hrs
3. 24 hrs a/f ~100% infected (by GFP change media) (0.5% HS; MEM (1 g/l)) (Cellgro 10-010 CM)
  - a. Wash 2 X PBS prior to change
4. Start oxidation ~18 hrs post serum starvation

**Media w/ 250  $\mu$ M oleate,  $\pm$  100 nM insulin, and <sup>14</sup>C-glucose (spiked, ~5.6 mM final)**

**Protein correction:** 1 ea. for protein quantification and correction  
Wash 2 X PBS  
Scrape in 1 ml RIPA+

**Oxidation:** 2 blank glucose w/o insulin  
2 blank glucose w/ insulin  
3 ea. glucose w/o insulin  
3 ea. glucose w/ insulin

Use 1  $\mu$ Ci per rxn. D-[U-<sup>14</sup>C]glucose (specific activity, 10 mCi/mmol; 1  $\mu$ Ci/ $\mu$ l)

### **Save media for std. curve 10 $\lambda$ , 1 $\lambda$ , and 0.1 $\lambda$ for glucose**

1. Change media of each flask
  - a. Add 100  $\mu$ l 1N NaOH to filter paper in cap
  - b. Remove cold media
  - c. Add HOT media then immediately cap w/ rubber stopper
  - d. Wait 2' btwn flasks
2. After 30' stop rxn.
  - a. Add 250  $\mu$ l 60% perchloric acid by syringe
3. Incubate  $\geq$ 10' w/ media on cells (to kill)
4. Allow to sit upright o/n @ 4°C
5. Prepare 20 ml glass scint. vials
  - a. Add 10 ml scint. fluid
  - b. Add 250  $\mu$ l 0.5 N NaOH to retain CO<sub>2</sub>
6. Transfer filter paper to 20 ml glass scint. vial
  - a. Vortex
7. Count