

13 May 2019

Hughes Lab

Harvesting C2C12s from culture for western blot analysis

Start to finish: Roughly 70 minutes

1. Aliquot the RIPA buffer into a 1.5 mL tube, specific amount is based on the surface area of the flask. Keep on ice until needed. See #4 below for more information.
2. Aspirate the media from the cell culture flask.
3. Wash the cells **2x** with **ice cold PBS** (maybe 3 mLs each time), aspirate away each time
4. Add **1 mL RIPA buffer** to the flask for every 75 mm<sup>2</sup> surface area of the flask bottom
  - a. Our TPP flasks are exactly 75 mm<sup>2</sup>
  - b. Ensure the **protease inhibitor** has been added to the RIPA buffer **immediately** before use!
5. Incubate flask on ice for **5 minutes**. Remove the flask from the hood and keep the ice outside of the hood at all times.
  - a. I repeat, keep the ice out of the hood.
6. After the 5 minutes, spray the flask with EtOH before returning to the hood.
7. Get a cell scraper to collect all the liquid and semiliquid fluid in one corner of the flask
8. Using a serological pipette, **collect all the contents** of the flask and transfer to a **15 mL Falcon tube**
  - a. Pipette up and down in the tube vigorously to help lyse the cells
  - b. Incubate the tube (about 1 mL of content) on ice for **30 minutes**. Timer starts now.
9. While the 30 minutes are going, remove everything from the hood and clean the tissue culture room properly. Everything else will be done at the lab bench.
10. Turn on the centrifuge and set the temperature to 4° C.
11. The Falcon tube has about 1 mL. For our purposes, we will divide the lysate into several **1.5 mL tubes** for storage in the freezer, however many you think you need. This will prevent unnecessary freeze-thaw cycles if it were all in the same tube.
12. Once samples are in their new tubes, return them to the ice as they are filled
13. Centrifuge the lysate in the **4° C centrifuge at 14,000g (rcf) for 15 minutes**
14. Collect the supernatant from each centrifuge tube and collect in a new tube
  - a. Keep one tube out to perform a **BCA Assay** if needed
  - b. Store others in the -20° C or -80° C freezer