

MITOCHONDRIAL RESPIRATION – SKINNED FIBER PROTOCOL

PREP

Up to one week before:

1. Weigh out all dry reagents for buffers A, B, C

Night before or morning of:

1. Make buffers
 - a. Add liquid reagents and pH buffer A
 - b. Add liquid reagents and BSA and pH buffer B
 - c. Add liquid reagents and BSA and aliquot buffer C into appropriate number for individual substrates. Add substrates to aliquots and mix then pH.
2. Prep for strip harvest (day of)
 - a. Label small petri dishes (2 per heart)
 - b. Label glass vials (3/heart): A, B, B
 - c. Thaw saponin on ice
 - d. Add buffer A to plates (6-10 mls, more in 2nd plate than the first) – keep on ice
 - e. Aliquot 3 mls buffer A to glass vials and 2ml to each buffer B vial – keep on ice
3. Weigh small pieces of foil for each planned strip – foil should be less than 1.5mg total. Label foil pieces with strip numbers

Harvest

1. Kill the mouse in CO₂ chamber and quickly cut out entire heart and immerse in 1st Buffer A dish on ice
 - a. Trim away vessels, fat and atria
 - b. Trim off the RV wall
 - c. Transfer to 2nd Petri dish on ice
 - d. Cut LV in half evenly starting at the apex
 - e. Using fine scissors make a small incision at apex of one half and cut a thin layer of endocardium working up from either side of the apex (Alternatively hold with one set of forceps and using second fine tipped forceps poke a path from the apex with incremental spreading to base of the heart then excise the two ends)
 - f. Repeat with the other half of the heart to obtain 2-4 strips (or 2 large that can be cut)
2. pull fibers apart using blunt insulin syringes – hold with one syringe and gently tease the fibers apart with the second (ideally in a motion perpendicular to the direction of the fibers). You should pull for approximately 20 minutes per strip and alternate between strips
3. Kill second mouse and harvest additional strips if planned
4. Add 3ul saponin to 3 ml of Buffer A in glass vial (vortex saponin vigorously before using)
5. put strips in glass vial with 3ml buffer A + saponin + micro stir bar for 30 min on ice in pipet tip lid on stir plate

MITOCHONDRIAL RESPIRATION – SKINNED FIBER PROTOCOL

6. transfer tissue and stir bar to glass vial with 2 ml of buffer B for 10 min stirring on ice
7. transfer tissue and stir bar to 2nd vial with 2 ml of buffer B for 10 min stirring on ice
8. Begin setting up and calibrating computer while strips are stirring

Strips can remain on ice in final vial of buffer B until ready for respiration

***Begin warming Buffer C + substrates in water bath – turned on from 25 degrees**

***Begin thawing injectates: ADP, cytochrome c, and oligomycin while strips are in Saponin**

COMPUTER

Connect the spectrophotometer via USB cable to computer

Open OOI Sensors

1. Dark calibration
 - a. occlude the blue light with forefinger
 - b. click DARK button
 - c. click STORE
 - d. release the blue light
2. Connect the spectrophotometer to the probe
3. Place end of probe in a large beaker
4. Stabilize with tape at the connection (2 places)
5. calibrate
 - a. oxy single temp
 - i. click the box for continuous
 - ii. place cursor in box under intensity
 - b. For Zero calibration
 - i. Make sodium hydrosulfite solution: approx 500ul of powder and 1 ml of respiration buffer in eppendorf tube – place in water bath
 - ii. Place probe end in eppendorf tube
 - iii. hit top green box – scan standard, wait a few minutes, the number in the intensity box should be relatively constant, then click scan standard again
 - c. For 100 calibration, type 100 in box under zero and put cursor in intensity box
 - i. WASH PROBE
 - ii. Place probe in first respiration buffer, wait a few minutes and click scan standard again
 - d. click curve fit
 - e. click update channel calls (hit OK)
 - f. file close

MITOCHONDRIAL RESPIRATION – SKINNED FIBER PROTOCOL

RESPIRATION

1. add 2 mls of buffer C (must be exact) to respiration chamber – make sure there are no bubbles
2. Turn chamber on
3. Prefill syringes with injectates (will inject 10ul but fill to 20 and inject down to 10 mark to assure no bubbles are injected)
4. place one strip and stir bar into chamber
5. put stopper on top with twisting, needing to push out any air. Air bubbles should blow out the top, then you can stop. Be careful not to allow liquid to flow out of the top
6. add fiber optic probe to chamber through tall metal opening. It should be reading approx. 100.
7. make sure probe is above stirrer
8. Start time chart (time on and scan on)
9. Wait until steady slope (approx 5 minutes) – If fast down slope then don't let go below 80
10. Stop scan and time chart
11. Inject ADP through tall metal port assuring you don't touch the probe OR through short white plastic but this is a tighter fit
12. Restart scan and time chart
13. Wait several minutes until stable slope
14. Repeat above steps with next injectates. Wait 10 full minutes after adding oligomycin to assure steady state
15. File save time chart – to disc
16. Save strip when finished on pre-weighed foil and keep in small glass beaker – dry overnight at 60°

17. To change strips
 - a. Rinse probe with H₂O bottle – NEVER USE ETHANOL
 - b. Rinse stopper vac dry.
 - c. Aspirate buffer from chamber and rinse chamber several times with water
 - d. Recalibrate for 100 intensity in next respiration buffer
 - e. Refill syringes, chamber, etc.