**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-7 kit optimization**

Using newest (not expired) kit.

**Sample preparation**

Kit lot number:

Kit expiration:

Date of experiment:

Prepare reagents

1. **Wash buffer**: add 10mL 20x Buffer to 190mL Mq H2O.
2. **Incubation buffer**: add 1mL 10x Blocking Solution to 9mL 1x wash buffer.
3. **Activity solution**: Place 30uL of coupler, acetaldehyde, NAD+, and reagent dye into 3380uL 1x Base Buffer.

Rat liver mitochondria (ab110346 positive control)

1. Prepare dilution (0.25 mg/mL) of mito suspension in incubation buffer.
   1. 0.25 mg/mL: 6 uL extract + 114 uL IB
   2. 0.1 mg/mL: 2.4 uL extract + 117.6 uL IB
   3. 0.05 mg/mL: 1.2 uL extract + 118.8 uL IB

A549 mitochondria (isolated in LM027-7, 20231129)

1. Prepare dilutions (0.25 mg/mL, 0.1 mg/mL, 0.05 mg/mL) of mito suspension in incubation buffer.
   1. 0.25 mg/mL: 29.6 uL extract + 90.4 uL IB
   2. 0.1 mg/mL: 11.86 uL extract + 108.1 uL IB
   3. 0.05 mg/mL: 5.93 uL extract + 114.1 uL IB

Lung lysates (human and mouse)

1. Give tissue a gentle squish in a Kimwipe to remove liquid from parenchyma and airways.
2. Weigh tissue. Aim for ~250mg tissue.
   1. Mass human tissue: \_\_\_\_\_\_\_
      1. Human number: \_\_\_\_\_\_\_
   2. Mass mouse tissue: \_\_\_\_\_\_\_
      1. Mouse number: \_\_\_\_\_\_\_
3. Place tissue in a Eppendorf SafeLock 1.5mL tube.
4. Eyeball an amount of 1mm glass disruption beads that looks like an equal volume to the tissue (v/v).
5. Pipet an equal volume of PBS to mass tissue (v/w) into the tube. (this represents 1000 mg tissue/mL PBS.)
6. Homogenize in bullet blender at speed 10 for 5min.
7. Place 100uL lysate + 400 uL EB in a tube.
8. Incubate on ice 20min.
9. Centrifuge 16000 x g 20min 4C.
10. Transfer supernatant to a new tube and discard pellet.
11. Prepare dilutions of lung lysate in incubation buffer.
    1. DF1: 120uL lysate
    2. DF2: 60uL lysate + 60uL IB
    3. DF4: 30uL lysate + 90uL IB
    4. DF8: 15uL lysate +105uL IB

**Assay procedure**

Assay

1. Add 100uL of each diluted sample per well.
2. Seal plate and incubate 3h RT on a 300RPM shaker. Did not have one available that could go this fast—put on orbital shaker at 250RPM
3. Aspirate each well and wash. Repeat for a total of two washes.
4. Blot plate on paper towel.
5. Place 220uL activity solution into PCR strip tubes in the same layout as the assay plate.
6. Multichannel 200uL Activity Solution to each well. (multichanneling ensures the wells start as close to each other as possible.)
7. Pop bubbles and immediately read plate as follows:
   1. Mode: Kinetic
   2. Wavelength: 450nm
   3. Time: 120min
   4. Interval: 1min
   5. Shaking: shake between readings

Draw plate layout below: