**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-6 kit validation**

**Sample preparation**

Do for all three kits.

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**: dissolve NAD+, Coupler, and Reagent dye in 250uL Mq H2O each. Place 20.83uL of each and also 100X acetaldehyde into 2 mL 1x Base Buffer.

Rat liver mitochondria (ab110346 positive control)

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: 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

Lung lysates (human and mouse, from LM027-1)

1. Samples are homogenized at 100 mg/mL tissue.
2. Place 100uL lysate + 400 uL EB in a tube.
3. Incubate on ice 20min.
4. Centrifuge 16000 x g 20min 4C.
5. Transfer supernatant to a new tube and discard pellet.
6. Prepare dilutions of lung lysate in incubation buffer.
   1. DF1: 120uL lysate
   2. DF4: 30uL lysate + 90uL 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. Add 200uL Activity Solution to each well.
6. 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: