**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-10 testing cell lysate**

Using newest (not expired) kit.

**Sample preparation**

Kit lot number:

Kit expiration:

Date of experiment:

I grew up T150 flasks of A549 and IMR90 cells. I have not treated them with anything. The objective of this experiment is to establish a working protocol for running cell lysates on the ALDH2 activity assay.

Prepare reagents

1. **Wash buffer**: add 10mL 20x Buffer to 190mL Mq H2O.
2. **Extraction buffer**: add 20uL PICIII, 2uL NaF, and 10uL NaOrt to 2mL Extraction Buffer.
3. **Incubation buffer**: add 1mL 10x Blocking Solution to 9mL 1x wash buffer. **Make 1mL**
4. **Activity solution**: Place 65uL of coupler, acetaldehyde, NAD+, and reagent dye into 6240uL 1x Base Buffer. **Make 2mL**

Prepare Rno liver mito control

1. Prepare dilution of mito suspension in incubation buffer.
   1. 1.25 mg/mL: 6uL extract + 114uL IB

Prepare cell lysates

1. Trypsinize confluent cells from T150 flask and resuspend in 25mL media.
2. Count cells.
   1. Total number of A549 cells:
   2. Total number of IMR90 cells:
3. Equate cell number to approximate mass total protein:
   1. Approximate total protein A549:
   2. Approximate total protein IMR90:
4. Spin cells 6min 1250RPM RT (big TC centrifuge) to pellet.
5. Wash cells 2x with ice-cold PBS.
6. Resuspend cells at 20E6/mL in extraction buffer +PI.
   1. Volume EB A549:
   2. Volume EB IMR90:
7. Incubate on ice 20min.
8. Centrifuge 16000 x g 4C 20min.
9. Transfer supernatants to a new tube.
10. Prepare dilutions of lung lysate in incubation buffer.
    1. DF1: 120uL lysate
    2. DF2: 60uL lysate + 60uL IB
    3. 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. 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:

|  |
| --- |
| A549 DF1 |
| A549 DF2 |
| A549 DF4 |
| IMR90 DF1 |
| IMR90 DF2 |
| IMR90 DF4 |
| Rno mito |
| IB blank |