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

**Sample preparation**

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.

Fractionated mitochondria

1. Fractionate A549 mitochondria according to ab65320 Mitochondria/Cytosol Fractionation Kit (abcam).
2. Pellet the sample.
3. Solubilize pellet by adding 9 volumes of Extraction Buffer.
4. Incubate on ice 20min.
5. Centrifuge 16000 x g 20min 4C.
6. Transfer supernatants to a clean tube and discard pellets.
7. Prepare BCA sample and perform BCA assay. (does not impact loading—can do during plate incubation)
8. Lysate aliquots can be stored long-term at -80C. Do not freeze-thaw.
9. Prepare dilutions (DF1, DF2, DF4) of mito suspension in incubation buffer.

Recombinant ALDH2

1. Prepare 5 tubes labeled 100%, 50%, 25%, 12.5%, and 0.
2. Place incubation buffer in tubes (100% = 240uL, 505 = 100uL, 25% = 80uL, 12.5% = 55uL, 0% = 150uL). **0% represents blank/negative control.**
3. Place 0.671uL recombinant ALDH2 (MyBioSource cat# MBS143867, activity = 0.14U/mL) in tube 100% and mix thoroughly (do not vortex).
4. Pipet 100uL of 100% into 50%. Mix well (do not vortex).
5. Pipet 80uL of 505 into 25%. Mix well (do not vortex).
6. Pipet 55uL of 25% into 12.5%. Mix well (do not vortex).
7. Place all tubes on ice.

**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: 30-120min
   4. Interval: 20s-1min
   5. Shaking: shake between readings

Draw plate layout below:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | ALDH2 dil1 |  |  |  |  |  |  |  |  |  |  |
| B | ALDH2 dil2 |  |  |  |  |  |  |  |  |  |  |  |
| C | ALDH2 dil3 |  |  |  |  |  |  |  |  |  |  |  |
| D | ALDH2 dil4 |  |  |  |  |  |  |  |  |  |  |  |
| E | mito dil1 |  |  |  |  |  |  |  |  |  |  |  |
| F | mito dil2 |  |  |  |  |  |  |  |  |  |  |  |
| G | mito dil3 |  |  |  |  |  |  |  |  |  |  |  |
| H | BLK |  |  |  |  |  |  |  |  |  |  |  |