**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM044-1 testing Alda-1 on various sample types**

Kit lot number:

Kit expiration:

Date of experiment:

Prepare reagents

1. **Wash buffer**: add 10mL 20x Buffer to 190mL Mq H2O. **Make 10mL**
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 20uL of coupler, acetaldehyde, NAD+, and reagent dye into 1920uL 1x Base Buffer. **Make 2mL**

**Prepare lysates**

Prepare a PCR tube strip as follows using the directions below:

|  |
| --- |
| Purified ALDH2 |
| Purified ALDH2 |
| Mito extract |
| Mito extract |
| Cell lysate |
| Cell lysate |
| Cell lysate |
| IB blank |

Rno liver mito

1. Prepare dilution of mito suspension in incubation buffer.
   1. 2.5 mg/mL: 24uL extract + 216uL IB
2. Place 120uL dilution into each of two PCR strip tubes.

Cell lysates

1. Trypsinize confluent cells from T75 flask and resuspend in 25mL media.
2. Count cells.
   1. Total number of A549 cells:
3. Equate cell number to approximate mass total protein:
   1. Approximate total protein A549:
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:
7. Incubate on ice 20min.
8. Centrifuge 16000 x g 4C 20min.
9. Transfer supernatants to a new tube.
10. Place 120uL of lung lysate in each of three PCR strip tubes.

Recombinant ALDH2

1. Prepare 3 PCR strip tubes. The first will be used to make the master mix. One of the remaining tubes will be for 10uM Alda-1 and the other will be for 40uM.
2. Place 299uL incubation buffer in the MM tube.
3. Place 0.9uL recombinant ALDH2 (MyBioSource cat# MBS143867, activity = 0.14U/mL) in the MM tube and mix thoroughly (do not vortex).
4. Aliquot 120uL MM into each of the two remaining tubes.

**Assay procedure**

Assay

1. Add 100uL of each sample per well.
2. Seal plate and incubate 3h RT on a 250RPM shaker (max speed that our WB shaker can go).

In the last 30min of the binding incubation, do the following steps:

1. Place 220uL activity solution into PCR strip tubes in the same layout as the assay plate.
2. Pipet Alda-1 or DMSO carrier into tubes 1-8 as follows:

|  |
| --- |
| 0.88uL DMSO |
| 0.88uL 10mM Alda-1 |
| 0.88uL DMSO |
| 0.88uL 10mM Alda-1 |
| 0.88uL DMSO |
| 0.44uL Alda-1 |
| 0.88uL 10mM Alda-1 |
| NA |

1. When binding incubation finishes, aspirate each well and wash. Repeat for a total of two washes.
2. Blot plate on paper towel.
3. Bring multichannel with tips, activity solution strip, and plate to plate reader. Put plate on plate reader tray.
4. Multichannel 200uL Activity Solution to each well. (multichanneling ensures the wells start as close to each other as possible.) Don’t get bubbles in the wells.
5. Pop any 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:

|  |
| --- |
| Purified ALDH2 +veh |
| Purified ALDH2 +Alda-1 **40uM** |
| Mito extract +veh |
| Mito extract +Alda-1 **40uM** |
| Cell lysate +veh |
| Cell lysate +Alda-1 **20uM** |
| Cell lysate +Alda-1 **40uM** |
| IB blank |