**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM051-5 testing IPF lung**

**I have donor lung pieces from the Schwartz lab at Anschutz. They gave me normal, transitional, and fibrotic tissue from the same lung. In LM051-1 and LM051-2, I had wide variability between technical replicates. In LM051-3 and -4, I showed that Dounce homogenization with 1% NP-40 following PBS homogenization decreases the spread of the data by about 90%. Now I will retry the experiment in LM051-1 and -2 with the improved sample prep protocol.**

**Human donor info:**

Kit lot number:

Kit expiration:

Date of experiment:

Prepare reagents

1. **Wash buffer**: add 500uL 20x Buffer to 9.5mL Mq H2O.
2. **Incubation buffer**: add 100uL 10x Blocking Solution to 900uL 1x wash buffer. **Make 1mL**
3. **Activity solution**: Place 40uL of coupler, acetaldehyde, NAD+, and reagent dye into 3840uL 1x Base Buffer. **Make 4mL**
4. **PBS + PI:** for each 1mL PBS, add 10uL PICIII, 1uL NaF, and 5uL NaOrt. **Make 3mL** (30uL PICIII, 3uL NaF, 15uL NaOrt, and 2952uL PBS)
5. **EB +PI:** same ratio as PBS +PI. **Make 2mL** (20uL PICIII, 2uL NaF, 10uL NaOrt, 1968uL EB)

Rno liver mito

1. Prepare dilution of mito suspension in incubation buffer.
   1. 5 mg/mL: 48uL extract + 192uL IB
2. Place 120uL dilution each into PCR strip tubes.

Lung lysates (human)

1. Prepare a pea-sized piece of “normal” tissue. Mince in PBS to remove blood and gently squish between two Kimwipes to remove excess PBS. Weigh tissue.
2. Place in a tube with 1.5 volumes (w/v) PBS +PI and mince to a pulp with scissors. Add 1mm glass beads. Homogenize in bead beater 5min max speed 4C.
3. Transfer 300uL PBS-homogenized tissue into an ice-cold Dounce homogenizer with 3uL NP-40. Homogenize in two fractions with 25-50 strokes, until mixture is completely smooth. Be careful not to generate a diabolical foam!
4. Transfer homogenized lysates into new tubes. Spin briefly in mini centrifuge to remove suspended bubbles.
5. Place 150uL of each lysate + 600 uL EB in two tubes.
6. Incubate on ice 20min.
7. Centrifuge 16000 x g 20min 4C.
8. Transfer supernatant to a new tube and discard pellet.
9. BCA assay the samples:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Conc from BCA ug/uL** | **ug input** | **vol lysate** | **vol IB** | **total volume** |
| normal |  |  |  |  | 450.00 |
| transitional |  |  |  |  | 450.00 |
| fibrotic |  |  |  |  | 450.00 |

1. Snap froze lysates in LN2 and stored at -80C O/N. Thawed on ice and continued with assay next day.
2. Prepare dilutions of lung lysate in incubation buffer.

**Assay procedure**

Assay

1. Add 100uL of each diluted sample per well.
2. Seal plate and incubate 3h RT on a 300RPM shaker.
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:

|  |  |
| --- | --- |
| normal\_rep1 | fibrotic\_rep1 |
| normal\_rep2 | fibrotic\_rep2 |
| normal\_rep3 | fibrotic\_rep3 |
| normal\_rep4 | fibrotic\_rep4 |
| trans\_rep1 | Rno\_mito |
| trans\_rep2 | Rno\_mito |
| trans\_rep3 | blank |
| trans\_rep4 | blank |