**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM051-3 testing homogenization process**

**I have donor lung pieces from the Schwarz 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. Anna from Abcam’s scientific support team suggested adding a dounce homogenization step after the PBS homogenization. I’ll test this on the existing normal homogenate to save sample.**

**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 18uL of coupler, acetaldehyde, NAD+, and reagent dye into 1728uL 1x Base Buffer. **Make 1.8mL**

Rno liver mito

1. Prepare dilution of mito suspension in incubation buffer.
   1. 5 mg/mL: 24uL extract + 96uL IB
2. Place 120uL dilution into PCR strip tube.

Lung lysates (human)

1. Using “normal” tissue homogenized in LM051-1.
2. Further homogenize 300uL PBS-homogenized tissue in an ice-cold Dounce homogenizer. Homogenize with 25-50 strokes, until mixture is completely smooth. This was harder than anticipated. I did around 30 strokes in a 500uL homogenizer. It was very difficult at first but eventually turned smooth. Want to use a bigger homogenizer (2mL) in the future.
3. Place 50uL each of Dounce-homogenized and non-Dounce lysates + 200 uL EB in two tubes.
4. Incubate on ice 20min.
5. Centrifuge 16000 x g 20min 4C.
6. Transfer supernatant to a new tube and discard pellet.
7. BCA assay the samples:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Conc from BCA ug/uL** | **ug input** | **vol lysate** | **vol IB** | **total volume** |
| un-dounced | 3.923 | 250 | 191.79 | 128.21 | 320.00 |
| dounced | 3.762 | 250 | 200.00 | 120.00 | 320.00 |

1. Snap froze EB samples in LN2 and stored O/N.
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:

|  |
| --- |
| undounce\_rep1 |
| undounce\_rep2 |
| undounce\_rep3 |
| dounce\_rep1 |
| dounce\_rep2 |
| dounce\_rep3 |
| Rnomito |
| blank |