**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM051-2 testing IPF lung**

**I have donor lung pieces from the \_\_\_\_\_ lab at Anschutz. They gave me normal, transitional, and fibrotic tissue from the same lung. I want to see if there’s a difference in ALDH2 activity in these different regions. Not doing any dilutions as I have not found that informative in the past.**

**Human donor info:**

Kit lot number:

Kit expiration:

Date of experiment:

Prepare reagents

1. **Wash buffer**: add 10mL 20x Buffer to 190mL Mq H2O.
2. **Incubation buffer**: add 100uL 10x Blocking Solution to 900uL 1x wash buffer. **Make 1mL**
3. **Activity solution**: Place 65uL of coupler, acetaldehyde, NAD+, and reagent dye into 6240uL 1x Base Buffer. **Make 6.5mL**

Rno liver mito

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

Lung lysates (human)

1. Give tissue a gentle squish in a Kimwipe to remove liquid from parenchyma and airways.
2. Weigh tissue.
3. Place tissue in a Eppendorf SafeLock 1.5mL tube.
4. Eyeball an amount of 1mm glass disruption beads that looks like an equal volume to the tissue (v/v).
5. Pipet an equal volume of PBS to mass tissue (v/w) into the tube. (this represents 1000 mg tissue/mL PBS.) I ended up adding a bit more PBS because the homogenate was too viscous to transfer and I’m controlling input by BCA anyway.
6. Homogenize in bullet blender at speed 10 for 5min. Did more like 25min each because tissue wouldn’t fully homogenize. In the future, finely mince the tissue with scissors before adding beads and homogenizing.
7. Place 200uL lysate + 800 uL EB in a tube.
8. Incubate on ice 20min.
9. Centrifuge 16000 x g 20min 4C.
10. Transfer supernatant to a new tube and discard pellet.
11. BCA assay the samples:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample** | **Conc from BCA ug/uL** | **ug input** | **vol lysate** | **vol IB** | **total volume** |
| normal | 4.22 | 783.9 | 185.76 | 264.24 | 450.00 |
| transitional | 2.226 | 783.9 | 352.16 | 97.84 | 450.00 |
| fibrotic | 1.742 | 783.9 | 450.00 | 0.00 | 450.00 |

1. 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. 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:

|  |  |
| --- | --- |
| 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 |