**ab115348 Mitochondrial ALDH2 Activity Assay Kit**

**Sample preparation**

Cell culture lysate

1. Add phosphatase inhibitors and protease inhibitors to extraction buffer prior to use. (per 1 mL buffer: 10 uL PICIII, 1 uL NaF, 5 uL NaOrt)
2. Scrape adherent cells to remove from plate.
3. Rinse cells twice with PBS.
4. Solubilize cell pellet at 2E7 cells/mL in kit Extraction Buffer.
   1. \_\_\_\_\_\_E6 cells/mL \* \_\_\_\_\_\_mL = \_\_\_\_\_\_**cells total**
   2. \_\_\_\_\_\_**cells total** / 20E6 cells/mL = \_\_\_\_\_\_**mL resuspension volume**
5. Incubate lysate on ice for 20min.
6. Centrifuge at 16000 x g 20min 4C.
7. Collect supernatant and discard pellet.
8. Prepare BCA sample and perform BCA assay.
9. Lysate aliquots can be stored long-term at -80C. Do not freeze-thaw.

Tissue lysate

1. Weigh tissue.
   1. Tissue mass = \_\_\_\_\_\_ mg
2. Mince tissue and thoroughly rinse in PBS to remove blood.
3. Add 500uL PBS per gram of tissue.
4. Homogenize tissue preferably using dounce homogenizer. Other methods may be used.
5. Collect BCA sample and run BCA assay.
6. Suspend homogenate to 25 mg/mL in PBS.
   1. [(\_\_\_\_\_\_ ug/mL from BCA \* \_\_\_\_\_\_ mL lysate) / 25 mg/mL] - \_\_\_\_\_\_ mL lysate = \_\_\_\_\_\_ mL diluent to add
7. Add 4 volumes of extraction buffer to a sample protein concentration of 5 mg/mL.
8. Incubate on ice for 20min.
9. Centrifuge at 16000 x g 20min 4C.
10. Transfer supernatant to a new tube and discard pellet.
11. Lysate aliquots can be stored long-term at -80C. Do not freeze-thaw.

**Assay procedure**

Prepare reagents

1. Wash buffer: add 20mL 20x Buffer to 380mL Mq H2).
2. Incubation buffer: add 6mL 10x Blocking Solution to 54mL 1x wash buffer.
3. Activity solution: dissolve NAD+, Coupler, and Reagent dye in 250uL Mq H2O each. Place 125uL of each into 12 mL 1x Base Buffer.
4. Dilute samples to desired concentration in 1x incubation buffer.

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. 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