**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-5 kit validation**

**Sample preparation**

Kit lot#:

Kit expiration:

Prepare reagents

1. **Activity solution**: Pipet 15.62uL NAD+, Coupler, 100X acetaldehyde, and Reagent dye into 1.5 mL 1x Base Buffer. Mix thoroughly.
2. **Incubation buffer**: add 100uL 10x Blocking Solution to 900uL 1x wash buffer.

Recombinant ALDH2

1. Prepare 5 PCR strip tubes labeled 100%, 50%, 25%, 12.5%, and 0.
2. Place incubation buffer in tubes (100% = 22.31uL, 50% = 11uL, 25% = 9uL, 12.5% = 6uL, 0% = 15uL). **0% represents blank/negative control.**
3. Place 0.685uL recombinant ALDH2 (MyBioSource cat# MBS143867, activity = 0.14U/mL) in tube 100% and mix thoroughly (do not vortex). **Be super careful not to get bubbles during the dilution curve.**
4. Pipet 11uL of 100% into 50%. Mix well (do not vortex).
5. Pipet 9uL of 50% into 25%. Mix well (do not vortex).
6. Pipet 6uL of 25% into 12.5%. Mix well (do not vortex).
7. Place all tubes on ice.

**Assay procedure**

Assay

1. Pipet 10uL of each dilution into a 96-well plate strip (uncoated, untreated).
2. Pipet 190uL activity solution into each well. Tap to mix and immediately proceed to plate read.
3. 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