**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-4 kit validation**

**Sample preparation**

Kit lot#:

Kit expiration:

Prepare reagents

1. **Activity solution**: Resuspend NAD+, coupler, and reagent dye in 250uL Mq H2O each if applicable. Pipet 20.83uL NAD+, Coupler, 100X acetaldehyde, and Reagent dye into 2 mL 1x Base Buffer. Mix thoroughly.
2. **Incubation buffer**: add 100uL 10x Blocking Solution to 900uL 1x wash buffer.
3. **Extraction buffer**: add 5uL PICIII, 0.5uL NaF, and 2.5uL NaOrt to 500uL EB.

Recombinant ALDH2

1. Prepare ALDH2 buffer (20mM Tris-HCl, 1mM DTT, 1 mM EDTA).
2. Prepare 5 tubes labeled 100%, 50%, 25%, 12.5%, and 0.
3. Place ALDH2 buffer in tubes (100% = 240uL, 50% = 100uL, 25% = 80uL, 12.5% = 55uL, 0% = 150uL). **0% represents blank/negative control.**
4. Place 0.671uL recombinant ALDH2 (MyBioSource cat# MBS143867, activity = 0.14U/mL) in tube 100% and mix thoroughly (do not vortex).
5. Pipet 100uL of 100% into 50%. Mix well (do not vortex).
6. Pipet 80uL of 50% into 25%. Mix well (do not vortex).
7. Pipet 55uL of 25% into 12.5%. Mix well (do not vortex).
8. 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