**ab115348 Mitochondrial ALDH2 Activity Assay Kit – LM027-3 kit validation**

**Sample preparation**

Do everything twice, once for each kit.

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.

Adherent cells

1. Spin down 1M cells (trypsinized leftovers from subculture) 500 x g 10min 4C.
2. Rinse cells 2x 5mL PBS. Spin 500 x g 10min 4C each time.
3. Resuspend pellet in 500uL extraction buffer + PI.
4. Incubate on ice 20min. Centrifuge at 16000 x g 4C 20min.
5. Transfer supernatant into a new tube and discard pellet.
6. Assay immediately or freeze -80C. Do not freeze-thaw.
7. Prepare dilutions:
   1. Label a PCR tube strip A-H.
   2. Place 10ul incubation buffer in tubes B-G. Place 20uL incubation buffer in H.
   3. Place 25uL cells + EB in tube A.
   4. Serially pipet 10uL of each tube into the next (eg. 10uL A into B, 10uL B into C, etc.), stopping at tube G.
   5. Final dilutions of sample in each tube in order should be: DF1, DF2, DF4, DF8, DF16, DF32, DF64, blank

**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

Draw plate layout below:

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | | 4 | 5 | 6 | | 7 | | 8 | | 9 | | 10 | | | 11 | | 12 | |
| A | A (old) | A (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |
| B | B (old) | B (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| C | C (old) | C (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| D | D (old) | D (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| E | E (old) | E (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| F | F (old) | F (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| G | G (old) | G (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |
| H | H (BLK) (old) | H (BLK) (new) |  |  | | | |  | |  | |  | |  | |  |  | |  | |  | |