**LM037-1: HNE assay (ab238538)**

Kit expiration:

Date of assay:

Kit lot number:

This is one of two ELISA assays performed in LM037-1.

**Standard preparation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **standard** | **vol diluent (uL)** | **vol std (uL)** | **source std** | **ug/mL HNE** |
| 1 | 320 | 80 | stock | 200 |
| 2 | 200 | 200 | std1 | 100 |
| 3 | 200 | 200 | std2 | 50 |
| 4 | 200 | 200 | std3 | 25 |
| 5 | 200 | 200 | std4 | 12.5 |
| 6 | 200 | 200 | std5 | 6.25 |
| 7 | 200 | 200 | std6 | 3.13 |
| 8 | 200 | 200 | std7 | 1.56 |
| 9 | 200 | 0 | NA | 0 |

**Sample preparation**

DF2, DF4, and DF8 are mitochondria isolated from A549 cells using Joe’s fractionation protocol. Buffer is the RIPA buffer alone. Rna mito is Abcam’s rat liver mitochondrial extract (ab110346).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **sample** | **vol diluent (uL)** | **vol sample (uL)** | **conc protein (mg/mL)** | **mass total protein (ug)** |
| DF2 | 55 | 55 | 7 | 385 |
| DF4 | 82.5 | 27.5 | 7 | 192.5 |
| DF8 | 96.25 | 13.75 | 7 | 96.25 |
| buffer | 55 | 55 | 0 | 0 |
| Rno mito | 55 | 55 | unk | unk |

Day 1 (allow 0.5h)

Reagent prep

1. Mix 16uL 100X Conjugate and 1584uL PBS.
2. Mix 16uL 4-HNE conjugate and 1584uL PBS. (final conc = 10ug/mL)
3. Mix diluted 4-HNE conjugate and 1X Conjugate Diluent at 1:1.
4. Add 100uL of mixture to each well and incubate 4C O/N.

Day 2 (allow 4h)

1. Remove all reagents from 4C and warm to RT.
2. Dilute 10X wash buffer in Mq H2O (need 50mL: 5mL 10X wash + 45mL H2O).
3. Wash plate 2x with PBS.
4. Add 200uL Assay Diluent to each well and block 1h RT.
5. Transfer plate to 4C until ready to use. Remove assay diluent from plate immediately before use.
6. Prepare standards and samples as directed above.
   1. If dilution of samples is needed, dilute in 1X PBS with 0.1% BSA (1mg BSA into 1mL PBS)
   2. Make sure samples are at room temp before use
7. Place 50uL standards/samples in respective wells.
8. Incubate 10 min RT on an orbital shaker. (speed is unspecified—record speed used here: \_\_\_\_\_)
9. During incubation, dilute anti-4-HNE antibody in assay diluent (need 1600uL. 1.6uL ab in 1499uL assay diluent).
10. Add 50uL anti-4-HNE antibody to each well.
11. Incubate 1h RT on an orbital shaker. Use same speed as recorded above.
12. In the last 5 minutes of incubation, dilute secondary-HRP in assay diluent (need 3mL. 3uL in 2997uL assay diluent).
13. Wash 3x with 250uL wash buffer. Aspirate after each wash.
14. After final wash, empty wells and tap strips on paper towel to remove excess wash buffer.
15. Add 100uL of secondary-HRP to each well.
16. Incubate 1h RT on an orbital shaker.
17. Wash 3x with 250uL wash buffer. Aspirate after each wash.
18. After final wash, empty wells and tap strips on paper towel to remove excess wash buffer.
19. Add 100uL substrate solution to each well.
20. Incubate at RT 2-20 minutes RT on an orbital shaker. If color changes rapidly, stop earlier.
21. Stop reaction by adding 100uL stop solution to each well.
22. Read immediately at 450 nm.

**Draw plate layout below:**