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| |  | | --- | |  | | Summary  **20240312**  I’m bored so I’m using a fun template for this protocol. Fun!  The purpose of this experiment is to determine whether I can get a concentration-dependent response from the two HNE ELISA kits I have (ab287803 and ab238538).  If one wins, I’ll move forward with developing it. If neither one appears to work, I suppose I’ll try again with HNE-adducted BSA as a “sample” instead. | | LM037-1 Testing HNE ELISA assays head-to-head Samples are A549 mitochondria isolated according to Joe’s protocol. I pelleted the mitochondria and then threw the pellet in LN2 for future resuspension. Buffer preparation **ELISA-compatible RIPA +SDS +PI**  Combine 500uL 1M Tris-HCl, 1540uL 1M NaCl, 100uL 10% SDS, and 5mL Mq H2O in a small graduated cylinder. Adjust pH to 7.3. Bring solution to 10mL with Mq H2O. Sterile filter 0.2uM. Phase 1: verify that I actually have pelleted mitochondria, or at least a pellet containing mitochondria.  1. Resuspend pellet in 3 packed pellet volumes **ELISA-compatible RIPA +SDS +PI**. Vortex to mix. 2. BCA lysate (see attached sheet). 3. Total protein concentration: \_\_\_\_\_\_\_\_\_ 4. Run a quick Western blot with a mitochondrial marker (see attached sheet).  Phase 2: test ELISA kits. Kit 1: ab238538 HNE assay   |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | A | std1 | std1 | std9 | DF8 |  |  |  |  |  |  |  |  | | B | std2 | std2 | std9 | DF8 |  |  |  |  |  |  |  |  | | C | std3 | std3 | DF1 | buffer |  |  |  |  |  |  |  |  | | D | std4 | std4 | DF1 | buffer |  |  |  |  |  |  |  |  | | E | std5 | std5 | DF2 | Rno mito |  |  |  |  |  |  |  |  | | F | std6 | std6 | DF2 | Rno mito |  |  |  |  |  |  |  |  | | G | std7 | std7 | DF4 |  |  |  |  |  |  |  |  |  | | H | std8 | std8 | DF4 |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  | |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | A | std1 | std1 | DF1 | buffer |  |  |  |  |  |  |  |  | | B | std2 | std2 | DF1 | buffer |  |  |  |  |  |  |  |  | | C | std3 | std3 | DF2 | Rno mito |  |  |  |  |  |  |  |  | | D | std4 | std4 | DF2 | Rno mito |  |  |  |  |  |  |  |  | | E | std5 | std5 | DF4 |  |  |  |  |  |  |  |  |  | | F | std6 | std6 | DF4 |  |  |  |  |  |  |  |  |  | | G | std7 | std7 | DF8 |  |  |  |  |  |  |  |  |  | | H | std8 | std8 | DF8 |  |  |  |  |  |  |  |  |  |   **Clear and prepare lysate**   1. Thaw lysate on ice. 2. Vortex very thoroughly. 3. Centrifuge 12,000 x g 10min 4C. 4. Transfer supernatant equally to two tubes. Record volume. Need 100uL each minimum. 5. Throw both tubes in LN2.   **Kit 1: ab238538 HNE assay** |