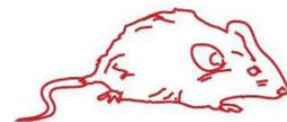


PROTEIN EXTRACTION FROM MOUSE HEART TISSUE USING THE MINILYS



Antibody Core, Cayman Chemical Company

CONTEXT

Cayman Chemical develops and produces antibodies intended for the research market. To ensure quality, suitable tissue lysates are needed to guarantee that our product meets or exceeds our quality control guidelines. As many of our targeted proteins are multi-pass transmembrane receptors, a more robust lysis system is often required.

MATERIALS

- Minilys homogenizer
- Lysing kits: CKMix 2mL (KT03961-1-009.2)
- Samples: 920 mg of mouse heart tissue
- Cell lysis buffer: 920 μ l of M-PER (Mammalian Protein Extraction Reagent, Pierce)

PROTOCOL

Minilys parameters: 5000 rpm, 4 cycles of 20 sec, 5 sec break in between cycles

Analysis:

- Total cytosolic and membrane proteins were extracted from the samples after homogenization on the Minilys.
- HEK 293 cells (transfected with S1P₃ or untransfected) were harvested off cell culture plates, followed by protein extraction.
- Total protein was determined using the BCA assay and the lysates were analyzed by western blot.

CUSTOMER



RESULTS

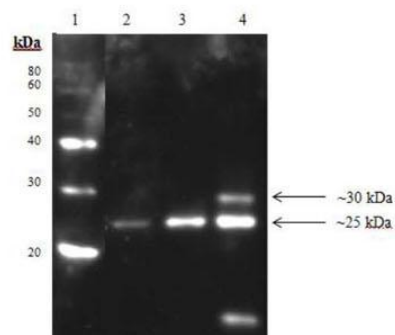


Figure 1: Western blotting using a S1P₃ polyclonal antibody (Sphingosine-1-phosphate Receptor 3). The additional band may represent post-translational modification. Lane 1: 3 μ L Magic Mark Protein Standard. Lane 2: 2.5 μ g of S1P₃ transfected HEK293 lysate. Lane 3: 5 μ g of S1P₃ transfected HEK 293 lysate. Lane 4: 50 μ g mouse heart lysate membrane fraction.

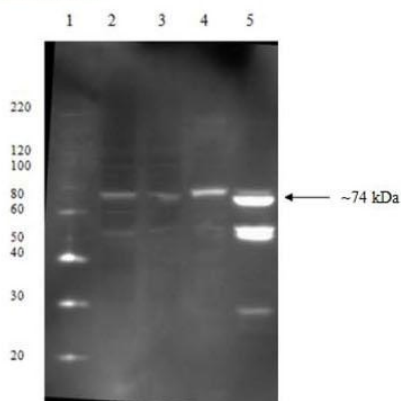


Figure 2: Western blotting using an Optineurin (C-Terminal) polyclonal antibody; Lane 1: 3 μ L Magic Mark Protein Standard. Lane 2: 60 μ g of HEK 293 cell lysate. Lane 3: 40 μ g of HEK 293 cell lysate. Lane 4: 50 μ g of mouse heart lysate cytosol fraction. Lane 5: Mouse heart lysate membrane fraction.

CONCLUSION

The **Minilys Homogenizer** is compact and user-friendly. The Minilys provided cleaner and more concentrated lysates, which gave more reproducible results for the target proteins analyzed. The **Minilys** can homogenize up to 3 samples at once in 2mL or 0.5 mL tubes, which is perfect for low-throughput experiments.

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