BEST PRACTICES IN PROTEINS SAMPLE PREPARATION WITH PRECELLYS® EVOLUTION

Proteins are the building blocks of life. They are vital to our existence and are found in every organism on Earth. As antibodies, enzymes or even hormones, proteins are implicated in chemical reactions, immunological processes and cell activities. Proteins also play a role in movement, structural support, storage, communication between cells, digestion and substances transportation. There is a huge variety of forms, sizes, shapes in proteins made from a long chain of these amino acids. Each type of protein has a stable and particular three-dimensional structure, which is determined by the order of the amino acids in its chain. This specific conformation is crucial and any change in shape can modify the function of the protein. That is why especially for thermo-sensitive molecules such as proteins, sample preparation is a critical step before proceeding to molecular down-stream analysis. The Cryolys® Evolution cooling unit combined with the Precellys® Evolution can be used to maintain a constant low temperature of 4°C within the Precellys® sample processing chamber.

IMPROVE EXTRACTION YIELDS OF PROTEIN FROM TISSUE USING THE PRECELLYS® RANGE

SUMMARY

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PRECIPITATION OF PROTEINS AND LIPID EXTRACTION FROM COMPLETELY HOMOGENIZED RAT SKIN TISSUES

/ CONTEXT

In this study, protein precipitation and bioactive lipid extraction from rat skin tissues is reported. The combination Solid Phase Extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach was used to measure concentrations of lipid mediators in these tissues. Rat skin tissues were homogenized using CKMix50_7mL Precellys® Lysing Kit and Precellys® Evolution tissue homogenizer combined with Cryolys® cooling unit. In addition, temperatures of homogenates were measured to investigate if temperature of complete homogenate of rat skin tissues remained below 40°C.

/ MATERIALS

- Automated Homogenizer: Precellys® Evolution and Cryolys® cooling unit
- Lysing Kits: Tissue grinding CKMix50_7mL (Cat #: KT03961-1-306.7)
- Tissue Samples: Rat skin tissues (dorsal and ventral hindpaw)
- Homogenization Buffer: Dry ice-cold methanol

/ PROTOCOL

Samples: A total of 20-90 mg of adult rat skin tissues (dorsal and ventral) were obtained following standard practice and stored at -80°C until use.

Homogenization: Tissues were homogenized in 500 µL methanol in 7 mL Precellys® tubes containing a mix of 2.8 mm and 5.0 mm ceramic beads. Tissues were homogenized by running 5 cycles of 10 sec at 8,000 rpm, with a 120 sec break between cycles.

Cryolys® cooling unit: Samples were homogenized once the temperature of cooling unit homogenization chamber reached 5°C.

Temperature measurements: Immediately after sample homogenization, sample temperatures were measured using temperature probe.

/ RESULTS

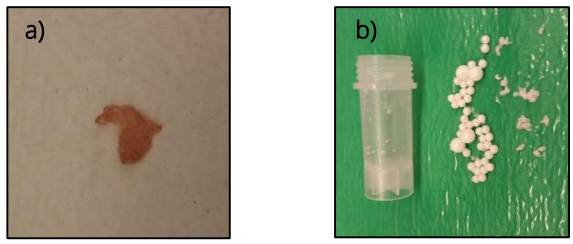

Fig. 1 Images of a whole rat hindpaw tissue sample (a), and homogenized tissue (b) using Precellys® Evolution.

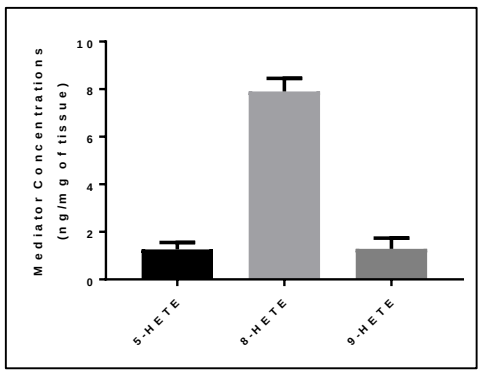

Fig. 2 Bioactive lipid mediators of the Arachidonic acid cascade were successfully measured, by LC-MS/MS, after lipids were extracted from the homogenate by solid phase extraction. Concentrations of 5-HETE, 8-HETE and 9-HETE were measured to be 1.3 ±0.3, 7.9±0.6 and 1.3 ± 0.4 ng/mg of tissue, respectively.

/ CUSTOMER

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SPPP-810-DU130

/ CONCLUSION

The rat hindpaw skin is considered to have thicker epidermis than the back skin, thus it is more challenging to obtain complete homogenate when working with these tissues. The combination of Precellys® lysing kit matrix and homogenization settings using Precellys® Evolution tissue homogenizer, allowed for the successful generation of a rat hindpaw whole tissue sample homogenate. The study also showed that Cryolys® cooling unit was able to maintain sample temperatures below 40°C.

PROTEIN EXTRACTION FROM MOUSE HEART TISSUE USING THE MINILYS

Antibody Core, Cayman Chemical Company

Precellys
The Science of Lysing

/ 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 µl 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 un-transfected) 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.

Watch the video on Youtube:

/ RESULTS

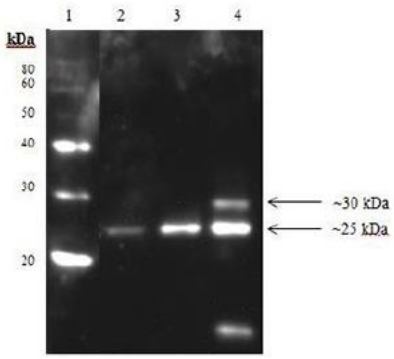
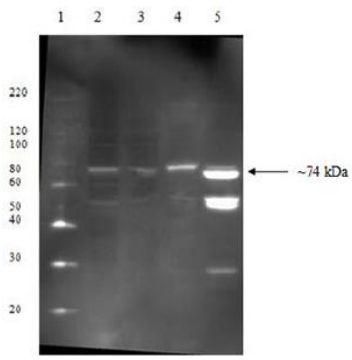


Figure 1: Western blotting using a S1P3 polyclonal antibody (Sphingosine-1-phosphate Receptor 3). The additional band may represent post-translational modification. Lane 1: 3µL Magic Mark Protein Standard. Lane 2: 2.5 µg of S1P3 transfected HEK293 lysate. Lane 3: 5µg of S1P3 transfected HEK 293 lysate.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

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



/ CUSTOMER

Cayman CHEMICAL

/ 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.

SPPP-810-DU101

SPECIFIC PROTOCOL FOR RNA EXTRACTION FROM MOUSE TISSUE

/ PROTEIN EXTRACTION FROM E.COLI FOR LARGE VOLUMES

• SAMPLE TYPE	E.Coli
• TARGETED MOLECULE KITS	Protein Extraction
• KITS	VKMix_7ml & VK05-15ml
• QUANTITY	5 & 10 ml of OD600 of 50
• PROTOCOL	7ml : 9000 rpm – 6 x 30s (60s break) 15ml : 9900 rpm – 6 x 60 s (60s break)
• INSTRUMENT	Precellys® Evolution

/ PROTEIN EXTRACTION FROM EYE TISSUES WITH 96 WELL-PLATE KITS

• SAMPLE TYPE	Neural retina & eye cup
• TARGETED MOLECULE	Protein extraction
• KITS	CKMix_WP
• QUANTITY	1
• BUFFER	50 µl M-PER Buffer
• PROTOCOL	For retina: 5500 rpm, 3x30s (30s break on ice) For eye cup: 8 200 rpm, 3x30s (30s break on ice)
• INSTRUMENT	Precellys® Evolution

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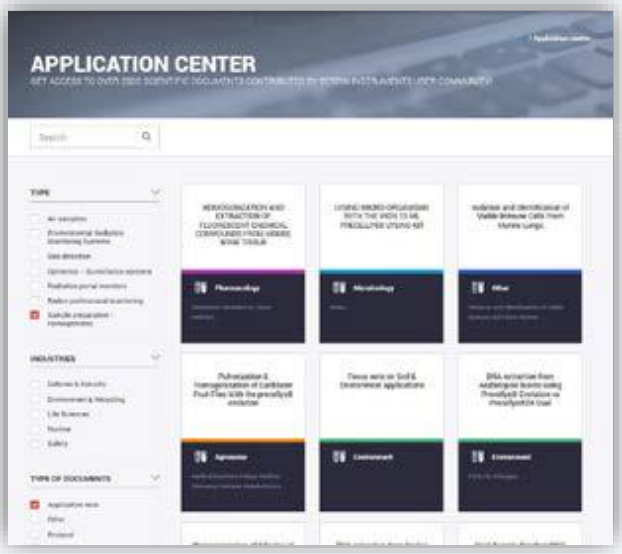

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0.5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with Cryolys® Evolution cooling unit