BEST PRACTICES IN PROTEINS SAMPLE PREPARATION WITH PRECELLYS® EVOLUTION

The preservation of proteins, whether on their primary, secondary or tertiary structure, is crucial in studies conducted on biological phenomena. Protein diversity is directly related to their varied shapes, sizes & interactions, conferring them functions ranging from simple structural roles to intercellular communication, storage, transportation and more. However, the degradation of proteins during their extraction modifies their properties and therefore the resulting analysis.

The Precellys® Evolution, combined with the Cryolys® Evolution as well as the homogenization kit 'Protein Safe', prevents this degradation. Tissue homogenization is optimized by maintaining a constant temperature between 0°C and 10°C during the process, to limit sample heating and thus protein denaturation, while the Protein Safe lysing kit inhibits the action of proteases released by the sample, responsible for the cleavage of the proteins.

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

SUMMARY

TISSUE EXTRACTED PROTEIN PROTECTION FROM ENDOGENOUS PROTEASES USING PROTEIN SAFE PRECELLYS LYSING KIT

/ CONTEXT

Homogenization of biological samples for protein extraction using Precellys® Evolution and Cryolys® Evolution ensures a high yield of protein recovery and prevents protein from heat degradation during this process. However, all proteins, including endogenous proteases, being protected during this step, a subsequent degradation of the proteins of interest can occur during and after the homogenization. Therefore, it is highly advised to protect proteins of interest from degradation by endogenous proteases released by the sample.
In this Application Note, we highlight how the use of the Protein Safe Precellys® Lysing kits ensuring an efficient protection of samples from endogenous proteases in different kinds of tissue homogenates.

/ MATERIALS

- Precellys® Evolution and Cryolys® Evolution
- Precellys Lysing kits CK14 (ref. P000912-LYSK0-A.0) and Protein Safe Precellys® Lysing kit CK14 (ref. P000973-LYSK0-A.0)
- Rat organs: liver, brain, heart, lung from Janvier labs
- Assay buffer (Potassium phosphate base buffer)
- Fluorescent substrate of proteases: universal protease substrate resorufin labelled (Sigma 11734334001)
- Fluorimeter PerkinElmer Type LS50B

/ PROTOCOL

The different tissue types (liver, brain, heart and lung) were cut off in pieces. 50mg of sample were then loaded in either CK14 Protein Safe lysing tubes or in regular CK14 Lysing tubes. Volume was then completed with 1,6mL of buffer. Tubes were placed on the Precellys® Evolution equipped with Cryolys® Evolution and processed using a generic homogenization program with the following settings:

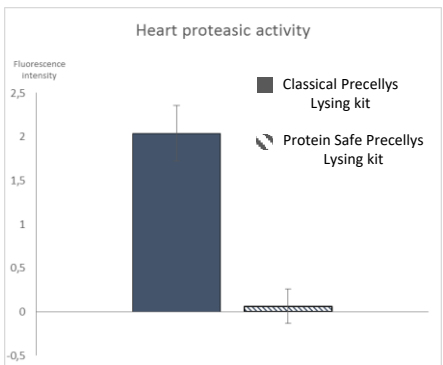
- 2 x 30sec at 6500rpm
- Break 15sec
- Cooling: 4°C.

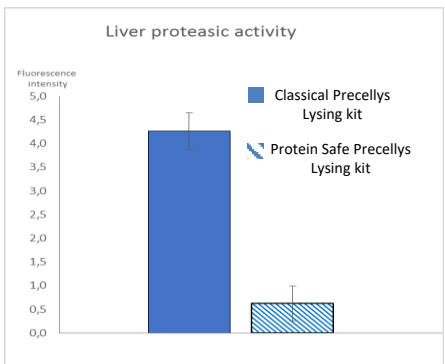
400µl of the obtained homogenate were then transferred in a new tube to proceed to proteases activity assay. Assay is based on incubation of the homogenate with a universal proteases resofurin labelled substrate. Active proteases degrade this substrate and release a fluorescent molecule. The level of fluorescence detected on the sample by fluorimeter is then related to protease activity. In case proteases are inactive (Protein Safe inhibitor), the reaction cannot occur and no or low fluorescence is observed.

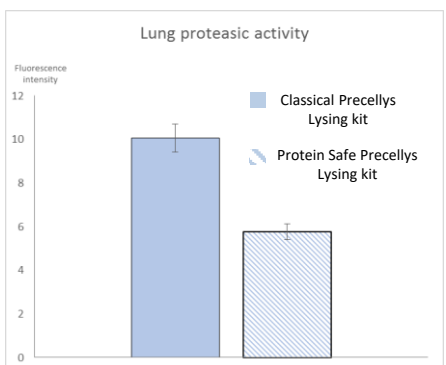
TISSUE EXTRACTED PROTEIN PROTECTION FROM ENDOGENOUS PROTEASES USING PROTEIN SAFE PRECELLYS LYSING KIT

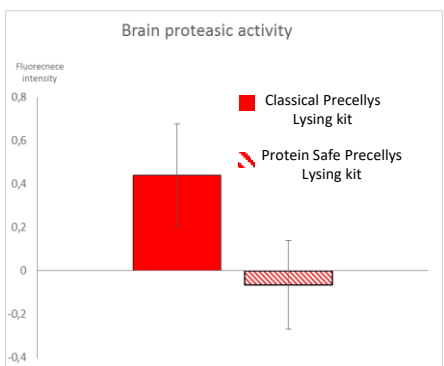
/ RESULTS

Fluorescence levels reflecting the proteases activity levels were measured in the different samples following the method described previously. The different activity levels measured for a same sample extracted with Classical Precellys® Lysing kit or the Protein Safe Lysing kit are represented in the figures 1 to 4 for Heart, Liver, Lung and Brain.









The protease activity significantly decrease when samples are homogenized using the Protein Safe Precellys® Lysing kits.

In the case of Heart, Liver and Brain the inhibition of proteases is close to 100% and in the case of Lung, close to 50%. The difference of efficiency can be explained by the expression levels of proteases in the different organs or by the nature of the protease themselves since Metalloproteases are not inhibited by the Protein Safe buffer (for MMP EDTA can be added to the tube but has not been evaluated in the present case).

/ CUSTOMER

/ CONCLUSION

The Protein Safe Precellys® lysing kit is efficient to inhibit proteases activity in various organs at a significant level during and after the homogenization process with Precellys® and Cryolys® Evolution. Proteins of interest are then protected from endogenous degradation once the homogenization step is finished.

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

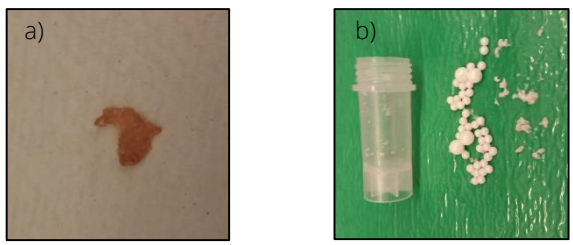


Fig. 1 Images of a whole rat hindpaw tissue sample (a), and homogenized tissue (b) using Precellys® Evolution.

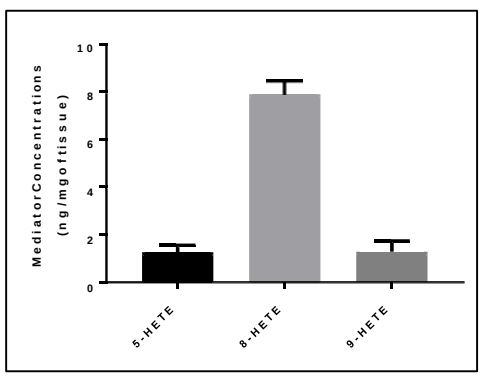


Fig. 2 Bioactive lipid mediators of the Arachidonic acid cascade were successfully measured, by LC-MS/MS, after lipids were extracted from the homogenate by solid phase extraction. Concentrations of 5-HETE, 8-HETE and 9-HETE were measured to be 1.3 ±0.3, 7.9±0.6 and 1.3 ± 0.4 ng/mg of tissue, respectively.

/ CUSTOMER

A.F. Domenichiello, B.C. Wilhite and C.E. Ramsden

Lipid Mediators, Inflammation and Pain Unit,

Laboratory of Clinical Investigation, National Institute on Aging

/ 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

/ 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

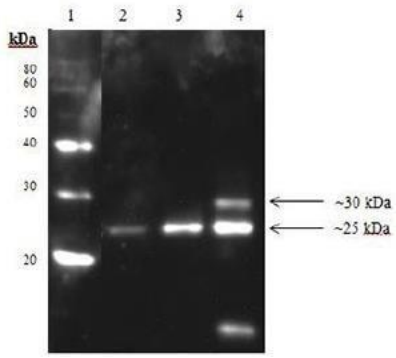
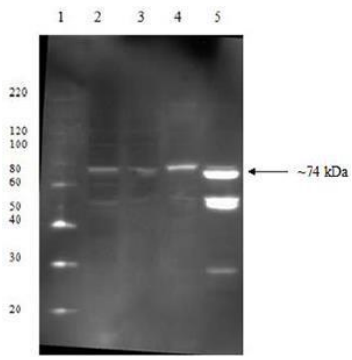


Figure 1: Western blotting using a S1P3 polyclonal antibody (Sphingosine-1-phosphate Receptor 3). The additional band may represent post-translational modification. Lane 1: 3µL Magic Mark Protein Standard. Lane 2: 2.5 µg of S1P3 transfected HEK293 lysate. Lane 3: 5µg of S1P3 transfected HEK 293 lysate.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

/ 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.

SPECIFIC PROTOCOL FOR RNA EXTRACTION FROM MOUSE TISSUE

/ PROTEIN EXTRACTION FROM E.COLI FOR LARGE VOLUMES

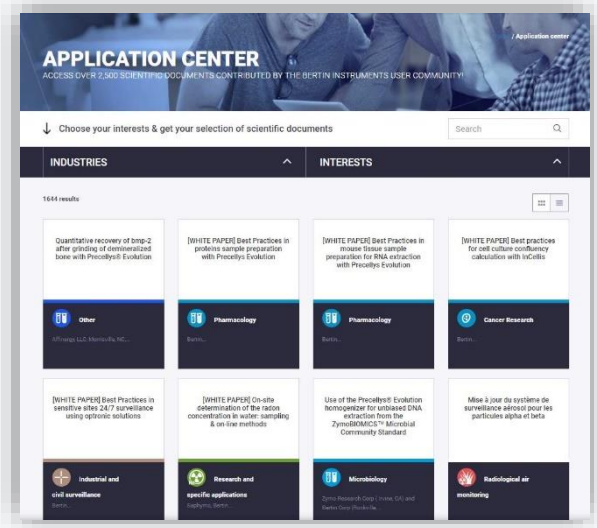
• SAMPLE TYPE	E.Coli
• TARGETED MOLECULE KITS	Protein Extraction
• KITS	VKMix_7ml & VK05-15ml
• QUANTITY	5 & 10 ml of OD600 of 50
• PROTOCOL	7ml : 9000 rpm – 6 x 30s (60s break) 15ml : 9900 rpm – 6 x 60 s (60s break)
• INSTRUMENT	Precellys® Evolution

/ 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

Use the Precellys® Application Center to find the appropriate protocol & optimize it with users feedback!

- **Find thousands of documents** presenting validated protocols
- **Find the appropriate kits**
- **Share with the Precellys® community**



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

Liste des produits **Nous contacter**

bertin INSTRUMENTS
CNIM group

Service client - commande : commande@ozyme.fr
Service technique :
Réactifs : 01 34 60 60 24 - tech@ozyme.fr
Instrumentation : 01 30 85 92 88 - instrum@ozyme.fr

OZYME
Des femmes et des hommes au service de vos recherches