EFFICIENT PREVENTION OF PROTEIN DEGRADATION BY PROTEASES WITH THE PROTEIN SAFE PRECELLYS LYSING KITS

CONTEXT

Homogenization of biological samples for protein extraction using Precellys Evolution and Cryolys Evolution ensures a **high yield of protein recovery** and **prevents protein degradation** from heat. Nonetheless, protein degradation can still occur as a result of the action of **endogenous proteases**, during and after the homogenization process. The use of **protease inhibitors** becomes hence key to ensure **successful protein extraction** for any type of downstream application.

In this Application Note, we highlight the use of the **Protein Safe Precellys Lysing kits, a** ready-to-use tool that contains an optimized **cocktail of protease inhibitors** to protect your samples from endogenous proteases in several types 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

CONTACT
Email: sample-prep@bertin-instruments.com
www.bertin-technologies.com

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PROTOCOL

- The different tissue types (liver, brain, heart and lung) were cut off into small pieces. 50 mg of sample were then loaded in both CK14 Protein Safe lysing tubes and in standard CK14 Lysing tubes. The volume was then completed with 1,6 mL of buffer. Tubes were placed on the Precellys Evolution equipped with the Cryolys® Evolution instrument, and processed using a generic homogenization program with the following settings:
    - 2 x 30sec at 6500rpm
    - Break 15sec
    - Cooling: 4°C.
- 400 µl of the resulting homogenate were then transferred into a new tube to proceed with the proteases activity assay. The 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. Fluorescence levels reflecting the proteases activity levels were measured in the different samples. If the proteases are inactive (Protein Safe inhibitor), the reaction cannot occur and no or low fluorescence is observed.

RESULTS

The different activity levels measured for each tissue sample extracted with the standard Precellys Lysing kit and the Protein Safe Precellys Lysing kit are represented in the figures 1 to 4.

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

The **inhibition of proteases was close to 100 %** for heart, liver and brain tissues. In the case of lung tissue, inhibition was close to 50%. The difference in efficiency can be explained by the expression levels of proteases among the different organs, or by the nature of the protease themselves. Matrix Metalloproteases (MMPs) are not inhibited by the Protein Safe buffer. For this particular case, EDTA can be added to the tube.

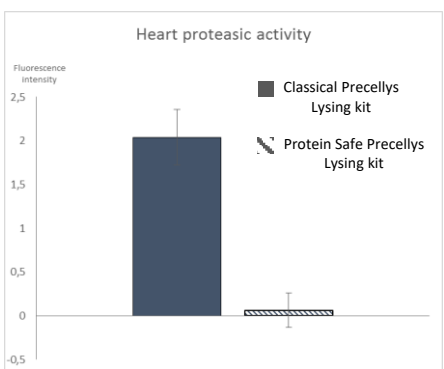

Figure 1

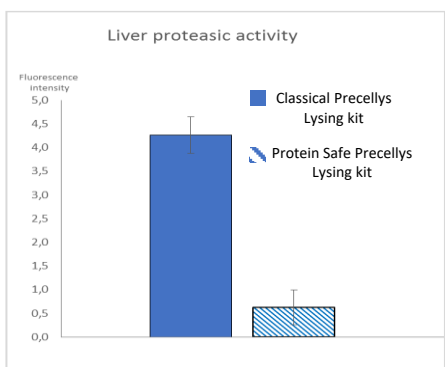

Figure 2

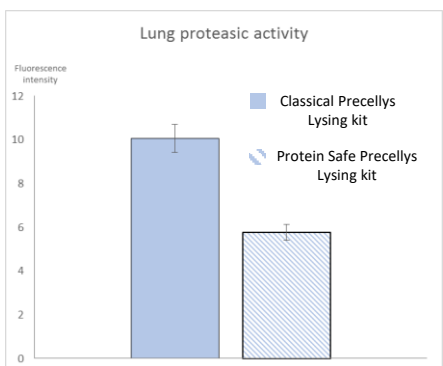

Figure 3

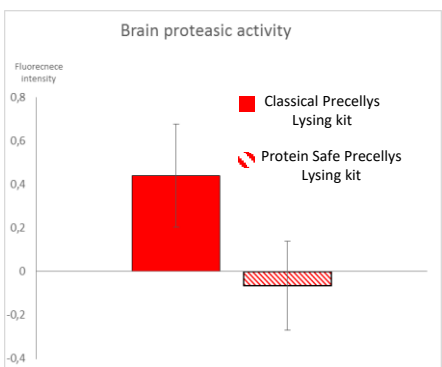

Figure 4

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Email: sample-prep@bertin-instruments.com
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CONCLUSION

The Protein Safe Precellys lysing kit is highly efficient in inhibiting proteases activity for several tissue types during and after the homogenization process with Precellys and Cryolys Evolution. Proteins of interest are then protected from endogenous degradation and allow to have access to a high-quality sample before downstream analysis.

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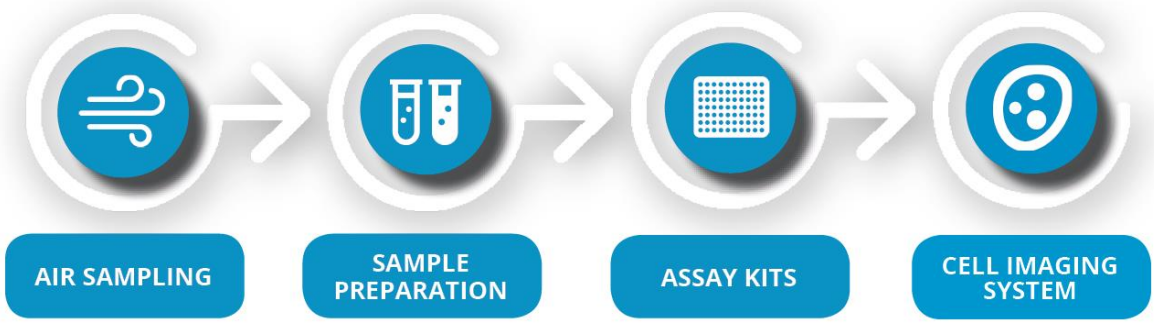

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