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

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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 ensures 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 (Sigma11734334001)
- Fluorimeter Perkin Elmer Type LS50B

**also compatible with Precellys Evolution Touch*

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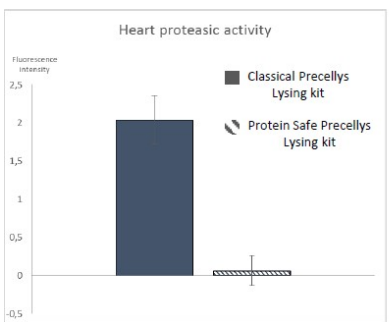
PROTOCOL

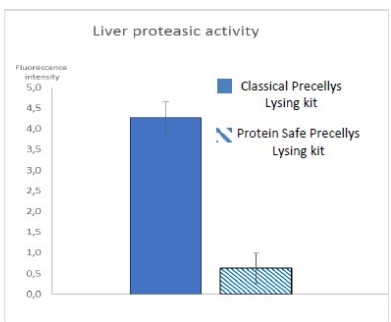
The different tissue types (liver, brain, heart and lung) were cut off in pieces. 50mg of sample were then loaded in either CK14 Protein Safe lysing tubes or in regular CK14 Lysing tubes. Volume was then completed with 1,6mL of buffer. Tubes were placed on the Precellys Evolution* equipped with Cryolys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

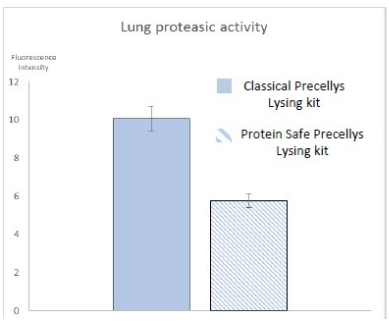
- 2 x 30 sec. at 6500rpm
- Break 15sec
- Cooling: 4°C.

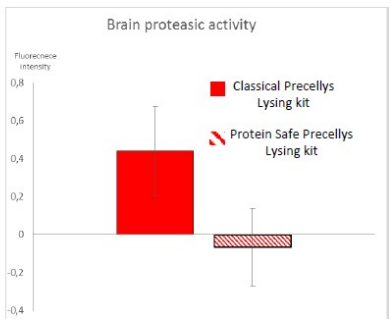
400µl of the obtained homogenate were then transferred in a new tube to proceed to proteases activity assay. Assay is based on incubation of the homogenate with a universal proteases resofurin labelled substrate. Active proteases degrade this substrate and release a fluorescent molecule. The level of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

RESULTS









Fluorescence levels reflecting the protease sactivity 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.

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The protease activity significantly decreases 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 proteases themselves, since Metalloproteases are not inhibited by the Protein Safe buffer (for MMPEDTA can be added to the tube but has not been evaluated in the present case).

CONCLUSION

The Protein Safe Precellys Lysing kit is efficient to inhibit protease sactivity 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 finished.