



BEST PRACTICES FOR CRYOGRINDING WITH THE PRECELlys EVOLUTION

Cryogrinding, also known as freeze powdering, cryogenic grinding, freezer milling, and cryomilling, is the act of turning a deep-frozen sample into a powder in a temperature-controlled environment. **Cryogenic grinding** is one of the most effective methods to extract DNA, RNA, proteins, and metabolites from a biological sample.

In biology, most analytical workflows require a first step of homogenization to **turn the sample into a liquid form compatible with the analysis technique**. This homogenization step is usually followed by an extraction step in which the molecule of interest – such as DNA, RNA, proteins, or metabolites– will be isolated. Sample homogenization plays a crucial role in the yield and quality of the extracted molecules. There are several techniques for homogenization that can be divided into chemical, physical, enzymatic, and mechanical disruption methods. Mechanical lysis is now widely acknowledged as the gold standard for the homogenization of biological samples. For this reason, Bertin Technologies has chosen the **3D bead-beating technology** to power its wide range of homogenizers, the Precellys tissue homogenizers.

An inadequate mechanical sample disruption protocol can lead to nucleic acid shearing or protein degradation. Proteins and RNA are thermosensitive molecules that can be degraded by the heat generated during mechanical homogenization. To address this challenge, Bertin Technologies has developed the Cryolys Evolution module, an **automated cooling system** that enables researchers to **control the temperature** of their samples during the whole homogenization process. To go even further, our team of scientists has designed **stainless steel metal lysing tubes** that allow users to **snap-freeze** their samples in liquid nitrogen prior to homogenization. In this white paper, we show how cryogrinding can be performed using the Precellys Evolution in combination with the Cryolys Evolution cooling module and the Precellys stainless steel metal tubes. We offer examples of **protocols to efficiently pulverize deep-frozen animal and vegetable samples** and maximize the recovery of nucleic acids, proteins, and metabolites.

DISCOVER OPTIMIZED HOMOGENIZATION PROTOCOLS FOR CRYOGRINDING

SUMMARY

Application note n°1: Freeze powdering using Cryolys® Evolution...../ Page 2

Application note n°2: Precellys Evolution: Whole mouse brain Cryogrinding...../ Page 3



FREEZE POWDERING USING CRYOLYS® EVOLUTION

M&S instruments Inc. OSAKA, JAPAN

/ CONTEXT

Grinding deeply-frozen biosamples or 'freeze-powdering' is one of the best ways to prevent high degradation of DNA/RNA or protein. In this report, we tested if the freeze-powdering can be performed with Precellys® Evolution equipped with Cryolys® Evolution on soft, hard and elastic samples.

/ MATERIALS

- Instrument : Precellys® Evolution & Cryolys® Evolution (P000062-PEVO0-A.0 & P000671-CLYS2-A.0)
- Kit : Stainless Metal tube (P000952-LYSK0-A.0)
- Beads: 2 x 6.8 mm ceramic beads (P000931-LYSK0-A.0)
- Liquid nitrogen for sample freezing
- Crushed dry ice for Cryolys Evolution
- Liver (swine, chicken), muscle (chicken, salmon, squid, shrimp), skin (chicken), whole deshelled clam, leaf(ginkgo, camellia), each.1g ca.

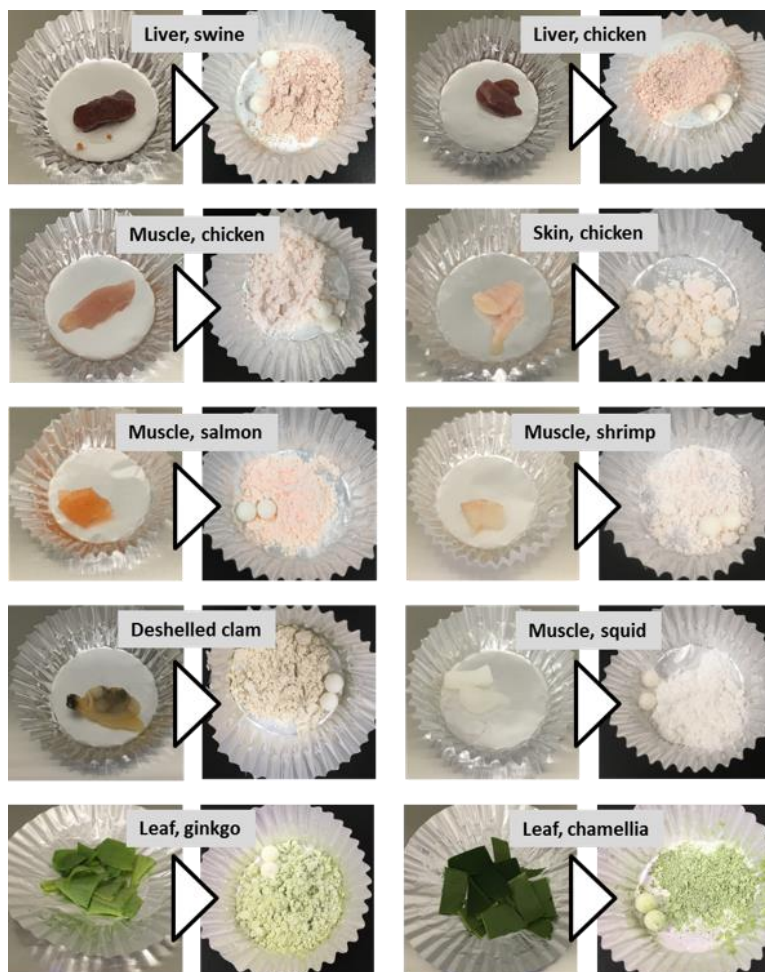
/ PROTOCOL

8800rpm, 1x10 sec.

Cooling temperature 0°C

The tubes, beads, and fresh samples were snap-frozen in the liquid nitrogen until just before grinding. Metal lids and screw part of tubes were kept untouched to the liquid nitrogen for preventing frost-locking.

/ RESULTS



/ CUSTOMER



工業入機器株式会社

The combination of Precellys® Evolution and Cryolys® Evolution is found to be a useful tool for freeze powdering of biosamples. All samples tested were completely ground. Except for chicken skin, the textures of the ground samples were like fine flour, meaning they were never thawed in and after the grinding process. The chicken skin became sticky because of the existence of a large quantity of fats. Results show not only animal organ or plants, but skin or squid muscle were completely ground meaning the freeze powdering by Precellys® Evolution is also useful for grinding elastic samples.



PRECELLYS EVOLUTION: WHOLE MOUSE BRAIN CRYOGRINDING

Metabolomics Platform (MEP) Faculty of Biology and Medicine University of Lausanne – Switzerland TEAV, T.

/ CONTEXT

Cryogrinding or Cryogenic grinding is an excellent method for tissue disruption. One advantage is that freezing the sample prevents degradation of metabolites such as RNA and proteins. Besides, hard and elastic samples that cannot be easily homogenized, can be rendered into powder using this technique. The step facilitates the access to different metabolites and their extraction by other analytical methods.

Here, we will show you how to perform cryogrinding using Precellys® Evolution, Cryolys® Evolution and metal tubes.

/ MATERIALS

- Instruments: Precellys® Evolution & Cryolys® Evolution (P000062-PEVO0-A.0 & P000671-CLYS2-A.0)
- Kit: Stainless Metal tube (P000952-LYSK0-A.0)
- Beads: 6.8 mm ceramic beads (P000931-LYSK0-A.0)
- Liquid nitrogen for sample freezing
- Dry ice for Cryolys® Evolution

/ PROTOCOL

A whole mouse brain and 6,8 mm ceramic beads were put into a Precellys® Metal tube, frozen in liquid nitrogen and cryogrinded with following parameters (one cycle only):

3 Beads – 10 seconds 8800 rpm

3 Beads – 5 seconds 8800 rpm

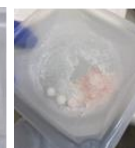
2 Beads – 5 seconds 8800 rpm

1 Bead – 5 seconds 8800 rpm

*For 5 seconds run, the Precellys® Evolution was stopped manually.

/ RESULTS

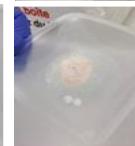
All the conditions tested were able to generate an homogeneous powder of the whole frozen mouse brain.



3 beads/10 sec



3 beads/5 sec



2 beads/5 sec



1 bead/5 sec

/ CUSTOMER

Tony TEAV MSc.
Metabolomics Platform (MEP)
Faculty of Biology and Medicine
University of Lausanne – UNIL
Rue du Bugnon, 19
CH-1005 Lausanne
Switzerland



The Precellys® Evolution is a very versatile homogenizer, its high disruption power allows to homogenize any kind of biological samples. The use of metal tubes, the Cryolys® Evolution and liquid nitrogen widen the possibilities of work and make it possible to perform cryogrinding.



Join the Bertin Instruments community!

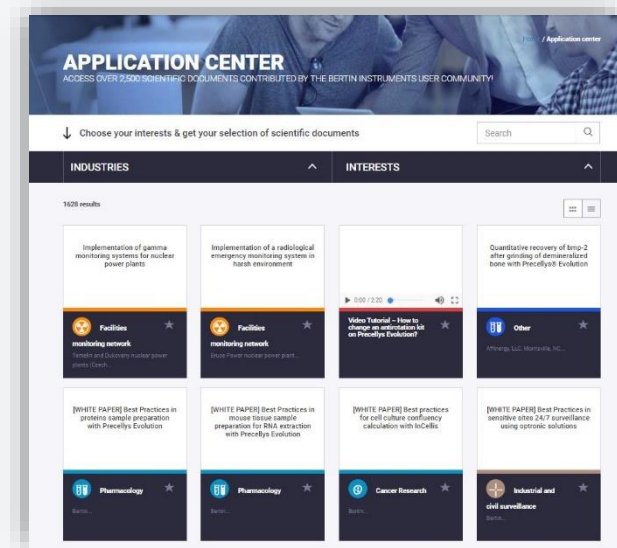
1500+ documents available online on the Application center

WWW.BERTIN-INSTRUMENTS.COM

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

- Find scientific documents
- Find the appropriate kit
- Share with the Precellys® community

<https://www.bertin-instruments.com/application-center/>



Precellys® Evolution & Cryolys Evolution, the most advanced homogenizer solution 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 the Cryolys® Evolution cooling unit

<https://www.bertin-instruments.com/products-range/sample-preparation-homogenizers/>