Precellys
The Science of Lysing

BEST PRACTICES FOR TOUGH SAMPLE GRINDING IN MOLECULAR BIOLOGY USING PRECELLYS®

The biggest challenge when working with fibrous, elastic and tough samples is efficient sample processing. The presence of elastic tissue, calcium phosphate (a rock-hard mineral) and other minerals can develop into a solid and tough matrix which can sometimes be difficult to homogenize.

Moreover, heat generation inside the lysing kit tubes during the process results in degradation and denaturation of these molecules such as proteins and RNA. Heat generation is even more severe when working with harder samples that require higher energy for efficient homogenization, resulting in a more challenging process.

The Precellys® Evolution homogenizer is capable of grinding hard samples within minutes thanks to its 3D-bead beating technology. When paired with the new Cryolys Evolution cooling unit, it also prevents heat generation inside the lysing kit tubes during the process, thus avoiding degradation and denaturation of thermo-sensitive molecules.

IMPROVE EXTRACTION YIELDS OF TOUGH SAMPLES USING THE PRECELLYS® RANGE

SUMMARY

HOMOGENIZATION AND EXTRACTION OF FLUORESCENT CHEMICAL COMPOUNDS FROM MOUSE BONE TISSUE

Atlanthera Laboratories, Saint-Herblain, France

/ CONTEXT

Atlanthera is a drug discovery company specialized in bone-targeted drug delivery through covalent binding of drugs to hydroxybisphosphonates (HBP), a class of molecule known for its high affinity to bone. Extraction of HBP-conjugated chemical from bone is challenging and require efficient tissue homogenization. The Precellys® Evolution system was tested for bone homogenization and compared with a cryogenic grinder.

/ MATERIALS

- Precellys® Evolution
- Precellys® lysing kit: MK28R_2mL (KT03961-1-008.2)
- Samples: Mouse tibia (50mg-110mg)
- Buffer: Phosphate-buffered saline (PBS)
- Extraction Buffer: 6N HCl, $CHCl_3$/methanol
- Suspension Buffer: Acetonitrile

/ PROTOCOL

Samples: Mouse tibia were removed, cleaned from soft tissues, snap frozen in liquid nitrogen and stored at -20 °C.

Homogenization: Frozen tibia were either dry processed or processed in PBS. Samples were homogenized by running 8 cycles of 10 sec at 8,500 rpm, with 10 sec break between cycles.

Analysis

Homogenized tissue was suspended in extraction buffer. Organic phase was purified, evaporated and the dry extraction was resuspended in acetonitrile prior to HPLC analysis.

/ RESULTS

The best homogenization efficiency was obtained at 8,500 rpm with a dry sample rather than homogenization with a buffer.

Compound extraction was equivalent to our standard protocol (cryogenic grinder).

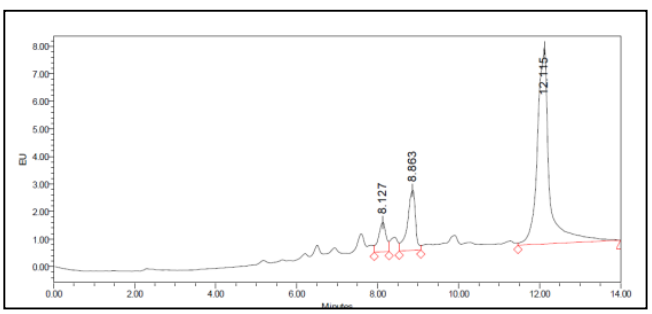


Figure 1. Example for HPLC analysis of compound extracted from bone tissue after homogenization with the Precellys® Evolution.

/ CUSTOMER

ATLANTHERA

/ CONCLUSION

Precellys® Evolution associated to the appropriate lysing kit is a suitable, simple and convenient homogenization system to extract chemical compounds from mouse bone tissue. Compared to our standard method (cryogenic grinder), Precellys® Evolution is much faster (shorter homogenization time, less manipulation steps and larger number of sample per cycle), and is free of cross-contamination between samples thanks to its single use consumables.

QUANTITATIVE RECOVERY OF BMP-2 AFTER GRINDING DEMINERALIZED BONE WITH PRECELLYS® EVOLUTION

Affinergy, LLC, Morrisville, NC, USA

/ CONTEXT

Bone Morphogenetic Protein 2 (BMP-2) is one of the most potent growth factors involved in bone growth and repair. Since it is present at low levels, its extraction and quantitation from bone is challenging. To evaluate the quantitative recovery of recombinant BMP-2 after a stringent grinding cycle in a Precellys® Evolution, we spiked commercial, demineralized cancellous bone with the protein, pulverized the samples, and measured the level of BMP-2 in the ground sample with an industry standard immunoassay.

/ MATERIALS

- Precellys® Evolution homogenizer
- Cryolys® Evolution cooling unit
- Precellys lysing kit: CK28R_2mL (KT03961-1-007.2)
- Samples: Human cancellous bone (50mg)
- Buffer: 4 M Guanidine Hydrochloride and 50 mM EDTA in Tris/HCl buffer pH 7.6 containing *Complete* protease inhibitor
- Recombinant BMP-2 from Medtronic INFUSE kit (7510600)
- Quantikine BMP-2 Immu7noassay kit (R&D Systems, DBP200)

/ PROTOCOL

Samples: 50mg of bone was spiked with 225ng of BMP-2.

Unspiked material was processed alongside the spiked one as a baseline control.

Homogenization: 1mL of extraction buffer was added to each 2mL Precellys® tube. Samples were ground by running 5 cycles of 20 sec at 5,000 rpm, with 30 sec break between cycles.

Cryolys® Evolution cooling unit: The temperature of the cooling unit was set to 4 °C during the grinding process.

Analysis

Grind samples were rotated at 8 rpm on a rotisserie shaker for 18h before being centrifuged at 10,000 rcf for 2 min. The supernatants were analyzed for BMP-2 content with a Quantikine assay.

/ RESULTS

The extract from the unspiked demineralized cancellous bone did not yield any signal in the Quantikine assay, indicating that its BMP-2 content was below the detection level of the assay.

For the extract of the spiked bone sample, the Quantikine assay measured a level of BMP-2 of 227 ng/ml, which corresponded to a **101% recovery of the BMP-2 spiked into the sample**.

/ CUSTOMER

AFFINERGY

www.affinergy.com

/ CONCLUSION

Stringent grinding of demineralized cancellous bone in guanidine hydrochloride extraction buffer using a Precellys® Evolution allowed to obtain the quantitation of BMP-2 by a standard immunoassay. Therefore grinding bone with a Precellys® Evolution instrument is a viable approach to pulverize the material for extraction of BMP-2 prior to immunoassay. The Cryolys® cooling unit ensured low temperature during the process which allowed to prevent protein denaturing.

TEETH SAMPLE GRINDING WITH 7ML METAL TUBES

/ CONTEXT

Until now, grinding very hard samples such as teeth and bones required a pre-grinding step in liquid nitrogen with hammer or mortar in order to obtain small sample particles . With the new stainless steel 7mL tube, Precellys® Evolution is capable of grinding up to 6 mammalian bone or teeth samples simultaneously without any pre-treatment. 7mL metal tubes offered a more sufficient and rapid sample preparation method when compared to manual grinding.

/ MATERIALS

- Precellys® Evolution homogenizer
- Cryolys® cooling unit
- Precellys lysing kit: 7mL stainless steel tubes (KT03961-1-602.M) containing 3 x 6.8mm ceramic beads (KT03961-1-107.BK)
- Samples: Cow marrowbone (200mg) and 1 Human teeth (1.12g) (premolar), dried for ~3 years
- Buffer: Dry grinding (no buffer was used)

/ PROTOCOL

Homogenization:

- Bone: 7,500 rpm, 10 x 20 sec (1 min break between cycles)
- Teeth*: 7,500 rpm, 1 x 20 sec

Analysis

DNA extraction by following standard DNA extraction protocol

*The whole teeth is placed into the metal tube and the metal tube is then cooled into liquid nitrogen prior the grinding process.

Metal tubes are reusable after cleaning with detergent solution and/or decontamination by autoclaving.

/ RESULTS

1) Metal tube (7mL) designed using inox steel

2) Metal tube adaptor

Marrowbone grinding

Dried premoral grinding

A fine powder of bone and teeth samples were obtained in a short time using the Precellys® Evolution.

/ CONCLUSION

The combination of 3D-grinding with the Precellys® Evolution and stainless steel tubes is an efficient method for very hard samples such as bones and teeth. In addition to the capability of processing 6 samples at once, the sample grinding time is dramatically reduced. The quality and quantity of extracted molecules is significantly increased.

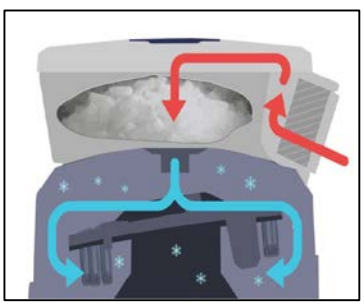
TECHNICAL INFORMATION
CRYOLYS ® EVOLUTION, PRECELLYS' NEW COOLING UNIT

When working with thermo-sensitive molecules, maintaining consistent low temperature during the homogenization eliminates severe heat-degradation. Bertin Instruments has developed the Cryolys® cooling unit for its Precellys® Evolution homogenizer, to maintain a low temperature during homogenization. Its exclusive technology relies on dry ice sublimation and ambient air offering an incredibly fast and accurate (Figure 1) cooling before, during and after sample grinding without frost generation.

/ TECHNOLOGY

The All-In-One unit: The Cryolys® Evolution is fully integrated to the cover of the Precellys® lid. No additional electrical source is required.

Dry Ice-based cooling: Up to 1.5kg of dry ice is added inside the Cryolys® container. Ambient air is aspirated into the Cryolys® container by fan, which is immediately cooled down to the desired temperature (0°C to 10°C). The cooled air is then diffused through the Precellys® Evolution lid into the chamber where samples are homogenized. The desired temperature is automatically regulated and maintained by the sensor installed inside the Precellys® lid.



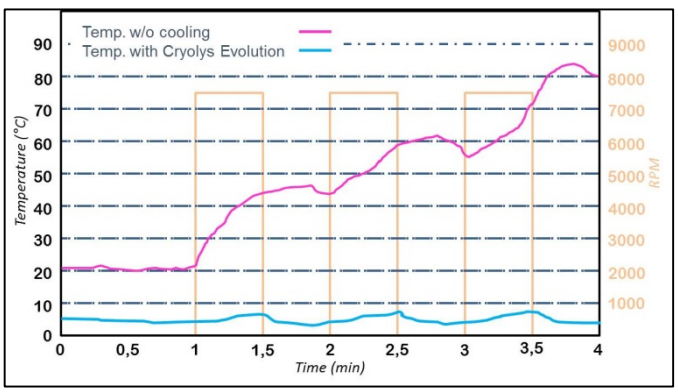


Figure 1. A comparison of temperature observed in the homogenization chamber with and without the cooling unit following 3 x 30 sec at 7,500 rpm, 30 sec break between each cycle protocol.

The best solution for the homogenization of thermo-sensitive samples

- Attachable cooling unit with no compressed air requirement, dry ice based
- Approximately 40 seconds to cool the air down from 26°C to 4°C
- Pre-set temperature range 0°C-10°C, with 1°C increments
- Live temperature monitoring

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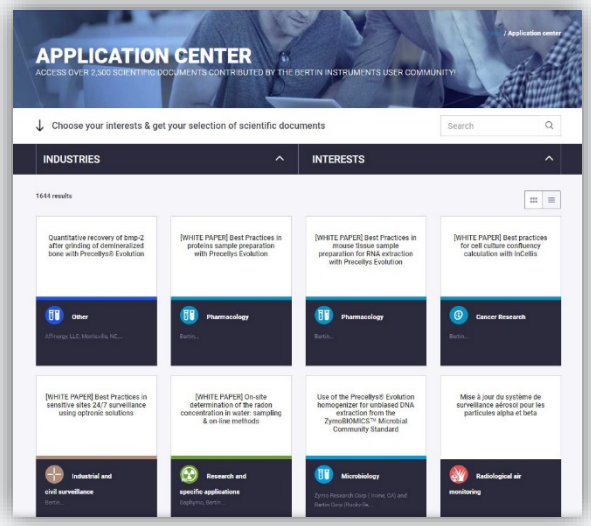

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **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