ApplicationForum

Perform efficient sample preparation with hard tumor tissue: Precellys® bead beating homogenizers solution

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The Precellys® Evolution is the most efficient homogenizer based on beadbeating technology. It gathers high flexibility and high efficiency for either routine or R&D projects. The instrument's unique figure-8 multi-directional motion provides the same high level of energy and homogenization efficiency to all the tubes.

The Importance of Sample Preparation

First step of sample preparation is critical to obtain the adequate quantity and quality of interest molecules from biological samples. It can be a delicate, technical and time consuming process. Bertin Technologies offers a range of robust, efficient and flexible homogenizers based on bead beating technology for grinding any tissues and lysing any cells prior to DNA, RNA and protein analysis. The Precellys® Evolution combined to dedicated lysing kit, is your best partner to save time and improve efficiency of your sample preparation protocol. The Precellys® Evolution (fig 1) has the capability to process 4 different sizes of tubes and up to 24 tubes simultaneously. Its flexibility and high efficiency gives scientists the ability to prepare any type of soft or hard sample from animal, human, plant or microorganism in seconds. The large range of Lysing kit, based on different beads matrix such as metal, ceramic and glass beads, make it particularly suitable for multiple biological applications.

Precellys® Evolution, a modern beat beating homogenizer

The Precellys® homogenizers move in a three dimensional, multidirectional motion, to effectively homogenize, lyse, or grind soft and hard tissue types in 30–60 seconds. The homogenizers utilize 35 unique lysing kits which consist of 0.5, 2, or 7, or 15 mL tubes filled with a variety of different lysing matrix beads specific to various types of soft or hard biological samples (fig 2). Ceramic (zirconium oxide) beads are well suited for animal or plant tissue, glass beads for microorganism lysis, and metal (stainless steel) beads for grinding hard materials such as bone, teeth, or hair. The tubes are DNase/RNase free and recommended for single use, which prevents cross-contamination, commonly observed with traditional methods. The homogenizers are also semi-automated and operator independent with programmable parameters such as speed, time, and cycle number. For thermosensitive applications such as enzymatic and metabolite extraction, the Cryolys® cooling unit can be coupled with the Precellys® homogenizer to keep the samples continuously cool at 4 °C, therefore preventing molecular degradation. The Precellys® homogenizers are available in high-throughput format for the processing of up to 24 samples simultaneously. The Minilys® homogenizer is suitable for lower throughput use and can process up to three samples simultaneously. For high-throughput sample processing, 200 samples can be processed in 30 minutes, which is an important time saving tool in any research laboratory.

Dedicated to Cancer Research

A major application for the Precellys® is tissue homogenization. Most soft tissue types such as liver, spleen or pancreas are relatively easy to homogenize and the CK14 (1.4 mm ceramic beads) or CKMix (mix of 1.4 and 2.8 mm ceramic beads) 2 mL tubes can be used in combination with a low Precellys® speed and short homogenization time [1]. However, hard tissues such as those derived from tumors can be particularly difficult to homogenize, and many cancer researchers utilize animal models such as mouse, rat, etc., to study cancer progression [2, 3]. Complete homogenization is needed to extract high quality DNA, RNA or protein for further downstream processing. For applications such as microarray or sequencing analysis, it is important to maintain reproducibility amongst biologi-

Figure 1. The Precellys® Evolution.

Figure 2. Mouse brains in a 15mL Precellys® tube before / after.

cal replicates during nucleic acid extraction. Banneau et al. [4] demonstrated that the Precellys® 24Dual increased the quality and integrity of total RNA extraction from cartilage tumor tissue compared to other bead/ball milling methods. Cartilage tumors (between 50 and to 100 mg of tissue) were resuspended in 1 mL of Trizol reagent and homogenized using the CK28R (2.8 mm ceramic beads) 2 mL tubes and a protocol of 6500 rpm, 3 cycles of 15 seconds each. For Precellys® protocols that require speeds >6000 rpm, the use of “R” or reinforced tubes are recommended to prevent tube breakage. RNA integrity was analyzed on Agilent’s 2100 Bioanalyzer (Fig. 3) and quality was classified using the ribosomal RNA ratio of 28S/18S and RIN (RNA integrity number), which are values assigned by the Bioanalyzer software. The RNA quality met the standards for downstream microarray analysis. For particularly thermosensitive RNA molecules, the Cryolys® cooling option can also be used to maintain a constant 4 °C temperature within the Precellys® chamber during sample homogenization.

A study by Lopez et al. [5] illustrates the use of the Precellys® for highthroughput sequencing after RNA extraction. This group sought to determine the p53 mutation pattern, a tumor suppressor gene commonly mutated in many cancer subtypes, and specifically in patients with sporadic colorectal carcinoma (CRC). Total RNA was extracted from 101 human colon or rectal tumor samples (between 50 and to 100 mg of tissue) using 1 mL of Trizol reagent and a Precellys® protocol of 6500 rpm for 1 cycle of 10 seconds. RNA quality and concentration was determined using an Agilent Bioanalyzer, followed by RTPCR and direct DNA sequencing to obtain the complete TP53 coding region. The results revealed the presence of 40 different p53 mutation types associated with CRC in a Brazilian cohort population, which were present in 54.5 % of the tumor samples analyzed. These studies demonstrate that the Precellys® homogenizers are the ideal tool for high-throughput and reproducible sample processing of tough to homogenize tissues derived from normal and malignant tumor subtypes.

Figure 3. BioAnalyzer profiles of total RNA and miRNA extracted from cartilage tumors using the Precellys 24 Dual. (a) represents “poor” RNA quality, (b) and (d) represents “medium” RNA quality and (c) and (e) represents “good” quality RNA extracts. Ranking is determined by the ribosomal 28S/18S ratio, RNA Integrity Number and Agilent 2100 BioAnalyzer profile. Adapted from “Homogenization of cartilage tumors to extract total RNA to microarray and sequencing analysis using Precellys bead beating technology” by G. Banneau et al., 2012, *BioTechniques*, 52, p. 196–197

Conclusion

Bead-beating technology significantly enhances extraction yields compared to traditional methods. The Precellys® lysing kits comprise of tubes prefilled with ceramic (zirconium oxide), metal (stainless steel), and glass beads, which are suitable for homogenizing soft or hard tissues. Bertin Technologies offers low and high-throughput homogenizers geared towards the needs of any R&D Research and Development laboratory, and significantly decreases sample preparation time. The Precellys® can efficiently homogenize hard to lyse sample types such as tumor tissues, bones, hair, and microorganisms, while maintaining reproducibility amongst biological replicates and eliminating cross-contamination.

Additional Information – References

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