



Performing efficient sample preparation with hard tumor tissue: Precellys® bead-beating homogenizer solution

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

The importance of sample preparation

The first step of sample preparation is critical for obtaining an adequate quantity and quality of molecules of interest 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 with a dedicated lysing kit, is a scientist's best partner

for saving time and improving the efficiency of sample preparation protocols. The Precellys® Evolution (**Fig. 1**) has the capability to process samples in four different sizes of tubes and up to 24 tubes simultaneously. Its flexibility and high efficiency give scientists the ability to prepare any type of soft or hard sample from animal, human, plant or microorganism tissue in seconds. The large range of lysing kits, based on different bead types such as metal, ceramic and glass, makes it particularly suitable for multiple biological applications.



Figure 1 | The Precellys® Evolution.

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APPLICATION NOTES

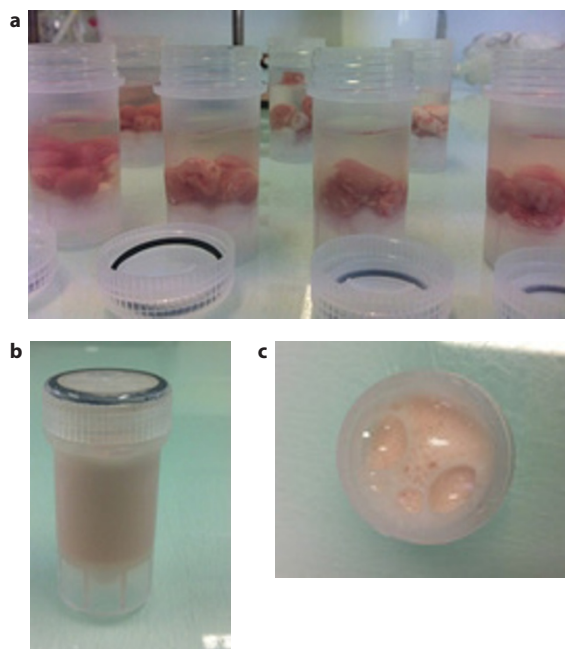


Figure 2 | Representative samples demonstrating the performance of the Precellys® Evolution. **(a)** Mouse brains in 15-ml Precellys® tubes before homogenization. **(b,c)** Homogenized samples in tubes viewed from the side **(b)** and the top **(c)**.

Precellys® Evolution, a modern bead-beating homogenizer

The Precellys® homogenizers move with three-dimensional, multidirectional motion to effectively homogenize, lyse or grind soft and hard tissue types in 30–60 s. The homogenizers use 35 unique lysing kits which consist of 0.5-, 2-, 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 hard materials such as bone, teeth and hair. The tubes are DNase and RNase free and are recommended for single use, which prevents the 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 thermo-sensitive applications such as enzyme and metabolite extraction, the Cryolys® cooling unit can be coupled with the Precellys® homogenizer to keep the samples continuously cooled at 4 °C, preventing molecular degradation. The Precellys® homogenizers are available in a high-throughput format for the processing of up to 24 samples simultaneously. The Minily® 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 min, making the Precellys® an important time-saving tool in any research laboratory.

Dedicated to cancer research

Most soft tissue types such as liver, spleen and pancreas are relatively easy to homogenize, and the CK14 (1.4-mm ceramic beads) and 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 for such samples¹. However, hard tissues such as those derived from tumors can be particularly difficult to homogenize, and many cancer researchers use animal models such as mice, rats, etc. to study cancer progression^{2,3}. Complete homogenization is needed to extract high-quality DNA, RNA or protein for further downstream processing. For an application such as microarray or sequencing analysis, it is important to maintain reproducibility among biological replicates during nucleic acid extraction. Banneau *et al.*⁴ demonstrated that the Precellys® 24-Dual increases the quality and integrity of total RNA extraction from cartilage tumor tissue compared to that obtained with other bead or ball milling methods. Cartilage tumors (between 50 and 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 three 15-s cycles at 6,500 r.p.m. For Precellys® protocols that require speeds of >6,000 r.p.m., the use of 'R', or reinforced, tubes is recommended to prevent tube breakage. RNA integrity was analyzed on Agilent's 2100 Bioanalyzer (**Fig. 3**), and quality was classified using the ratio of 28S/18S ribosomal RNA and the RIN (RNA integrity number), which are values assigned by the Bioanalyzer software. The RNA quality met the standards for downstream microarray analysis. For particularly thermo-sensitive RNA molecules, the Cryolys® cooling option can be used to maintain a constant 4 °C temperature within the Precellys® chamber during sample homogenization.

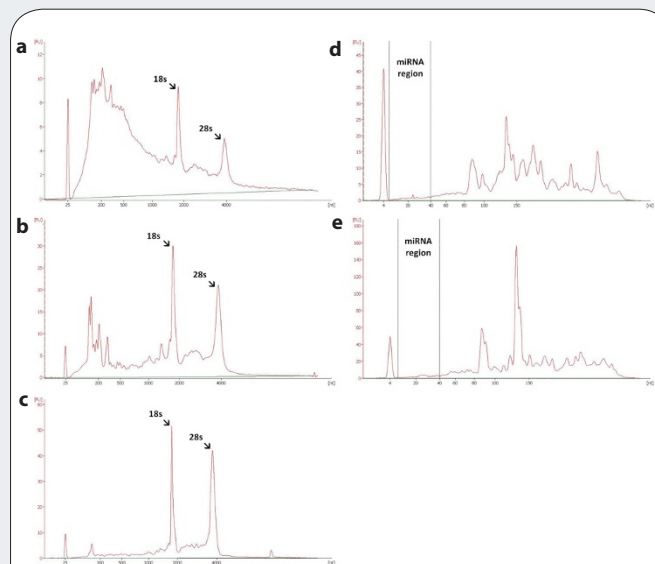


Figure 3 | Bioanalyzer profiles of total RNA and miRNA extracted from cartilage tumors using the Precellys® 24-Dual. **(a–e)** Graphs representing **(a)** 'poor' RNA quality, **(b,d)** 'medium' RNA quality and **(c,e)** 'good'-quality RNA extracts. Ranking is determined by the ribosomal 28S/18S RNA ratio, RIN and Agilent 2100 Bioanalyzer profile. Adapted from ref. 4.

A study by Lopez *et al.*⁵ illustrates the use of the Precellys[®] for high-throughput sequencing after RNA extraction. The group sought to determine the mutation pattern of *p53* (*TP53*), a tumor-suppressor gene commonly mutated in many cancer subtypes, and specifically in patients with sporadic colorectal carcinoma. Total RNA was extracted from 101 human colon or rectal tumor samples (between 50 and 100 mg of tissue) using 1 ml of Trizol reagent and a Precellys[®] protocol of one 10-s cycle at 6,500 r.p.m. RNA quality and concentration were determined using an Agilent Bioanalyzer, and then RT-PCR and direct DNA sequencing were carried out to determine the complete *p53* coding region. The results revealed the presence of 40 different *p53* mutation types associated with colorectal carcinoma 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.

Conclusion

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

1. Ason, B. *et al. Lipids* **11**, 991–1003 (2011).
2. Courtin, A. *et al. PLoS One* **8**, e67330 (2013).
3. Ehreleiter, K. *et al. Cancer Cell* **16**, 149–160 (2009).
4. Banneau, B., Ayadi, M., Aremenoult, L. & Carvalho, E. *Biotechniques* **52**, 196–197 (2012).
5. Lopez, I., Oliveira, L.P., Tucci, P., Alvarez-Valin, F., Coudry, R.A. & Marin, M. *Gene* **10**, 81–87 (2012).

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