Application Forum

A Major Step Towards Efficient Sample Preparation with Bead-Beating

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INTRODUCTION

Tissue homogenization is a key step for molecular biology studies. This first critical step of the long biological process is aimed at detecting or quantifying DNA, RNA, proteins, or drugs.

Blending, vortexing, mortar and pestle grinding, or hammering are traditional methods to disrupt the tissue matrix to liberate the nucleic acids or proteins. These methods are laborious and require training to operate. But more problematic, these methods are limited and are unable to follow the latest requirements of analysis equipment, which has radically improved in throughput (automation and parallelization), detection limits, and linearity.

Researchers involved in extraction process are looking for a homogenization method that combines simplicity and efficiency. Cross-contamination, target molecule recovery, and stability are important factors that affect the data quality and high-throughput (HTP) productivity.

Precellys®24 represents a hands-off approach to tissue sample preparation. Introduced three years ago by Bertin Technologies, the Precellys®24 bead beater is a uniquely designed equipment that uses small beads and a high-speed motion to grind, homogenize, or lyse tissues in 30 seconds.

PRINCIPLES OF THE TECHNOLOGY

Bertin Technologies has designed a new generation tissue homogenizer using bead-beating technology in 2 mL tubes. This system, Precellys®24 (Figure 1), allows homogenization of a large range of biological samples, from soft to hard and even elastic, simply by varying the bead type and speed.

A figure-8 multidirectional motion gives shaking energy to the beads that grind from 1 to 24 samples in individual sealed tubes, which ensures no cross contamination between the tests. Typical homogenization time is 30 seconds for a vast majority of samples. Nucleic acid or proteins are separated from the tissue matrix, and pipetted after a short centrifugation step to pellet the debris.

Bead-beating homogenization is optimal in 2 mL tubes, giving an excellent efficiency with powerful grinding forces. The protocols have to be adjusted so that the tissue is well homogenized without any degradation or denaturation of the target molecules. When compared to traditional homogenization methods, the Precellys®24 provides higher yields and intact DNA, RNA, and proteins. When the protocol is set up and validated, the sample preparation process remains the same with no bias in analysis, on a time basis, or between operators.

With the eight ready-to-use grinding kits, composed of 2 mL tubes pre-filled with beads, the Precellys has total flexibility. Ceramic beads will give the best results (yield and quality) on soft animal or vegetal tissue. Metal beads are dedicated to hard samples like

bones, teeth, and hair. Glass beads are adapted to mechanical lysis of cell or bacteria. A new reference with a mix of beads makes it possible to grind heterogeneous samples for extraction of genomic bacteria from a tissue.

For animal, vegetal, or microbiological samples, the Precellys®24 covers all the needs of homogenization and lysis in the laboratory.

High-throughput Precellys®24 homogenization fulfills the requirements of downstream analysis. It can handle 24 samples in one sample preparation step that lasts an average of 30 seconds. This means that processes can be very fast with 200 samples being homogenized in just 30 minutes. These individual tubes also make sample storage easy.

The major trend in downstream analysis is to work on small samples, not only to benefit from parallelization and cost reduction of the process but also because new technology analysis is now available. Some fragile tissues, such as rat or mouse lymph node and tumors, are very difficult to handle with mechanical methods. These tiny pieces must be manually ground, which is really time-consuming¹. Precellys®24 is able to grind samples as small as 1 mg.

For some proteomic methods, heat generation can be a concern. During motion, mechanical impacts between beads and sample generate heat. Operation in a cold room does not help because the temperature set at 4°C does not compensate for heat generation. Most often, the homogenization process is so rapid that the temperature does not reach denaturation levels. However, the optional Cryolys is a cooling adaptor for the Precellys®24, which converts it into the only bead beater with cooling during homogenization. Cold air (-50°C) is blown beside the tubes so that temperature increase is limited to 10°.

Figure 1: Sample tubes filled with beads are moving in a figure-8 multidirectional motion to grind from soft to hard tissues.

Figure 2: Animal protein extraction with Precellys®24 using the CK28 beads at 5,000 rpm - 1 cycle of 10 s. Higher protein extraction using Precellys®24 with > 0.1 g of animal tissue.

Figure 3: Bacterial protein extraction with Precellys®24 using VK05 beads at 5,500 rpm - 1 cycle of 30 s. Precellys®24 always extracts proteins.

COMPARISON WITH CONVENTIONAL METHODS

Precellys®24 was evaluated at the Natural Science Department of McPherson College², for the homogenization of animal (muscle) and plant tissues (wheat leaf) and bacteria (*E. coli*) in comparison with conventional methods. Extraction of proteins was performed. Tissue size varied from 0.01 g to 0.5 g. The appropriate kinds of beads were used for each tissue—big ceramic beads (ref. CK28) for animal and vegetal tissues, and glass beads (ref. VK05) for bacteria.

The conventional protocol for protein extraction consists of vortexing and icing. After Precellys®24 homogenization or vortexing, centrifugation was applied at 14,000 for 10 minutes. Spectrometry, SDS-PAGE and SEC-HPLC techniques were carried out to assess protein extraction.

Results of extraction are given in Figures 2-3. Precellys®24 extracts significantly higher amounts of protein vs. conventional methods ($p < 0.05$). With chromatography, Precellys®24 protein extracts exhibit distinctive protein peaks. Further work showed that the use of RIPA buffer for protein extraction also increased the quantity of proteins extracted up to 5-fold with a Precellys®24.

The process is fast, simple and reproducible with no degradation. Precellys®24 always extracts proteins with higher yield for the three samples. This study also shows the ability of Precellys®24 for mechanical homogenization of very small samples.

ADDITIONAL SPECIFIC APPLICATIONS

Pharmacology Labs:

Before mass spectrometry analysis, tissues are typically extracted by grinding in liquid nitrogen followed by the stepwise addition of solvents. Time-consuming and difficult to automate, the multiple steps can introduce bias in analysis. High throughput and standardization of the sample preparation with Precellys®24 directly improves extraction quality, meaning better yield and reduction of variability³. Single use tubes which are ready-to-use allow the user to save time in the sample preparation process.

Cancer Research:

Small samples are difficult to handle by mechanical blender. The 2 mL tube of Precellys®24 is the perfect size to homogenize tumor xenografts⁴. 24 tubes and fast protocols offer a high-throughput solution for large comparative studies on multiple kinds of samples.

Forensic and Crime Lab:

The Precellys®24 is able to grind hard samples like bones or nails. Traditionally the extraction of DNA from bones involved the maceration of compact bone followed by decalcification steps prior to the purification of DNA utilizing organic solvents. This lengthy procedure required up to two weeks to produce DNA. The Precellys®24 was used to optimize sample preparation and is included in a system to routinely obtain DNA profiles from bone within 48 hours⁵.

Cotton swabs can also be homogenized with Precellys®24 to increase detection limits and yield of DNA extraction with automated equipment⁶.

CONCLUSION

Precellys®24 is a suitable and reliable system for a wide range of tissues. This equipment provides an easy and reproducible isolation of stable RNA, active proteins and full-length genomic DNA. The Precellys®24 homogenization will also save hours of work in the sample preparation process so that it is no longer a limiting step, with increased efficiency on particularly challenging samples, such as small or tough ones. Visit our website to find user information, application forms, a user database, scientific publications and more.

LINK:

www.precellys.com

REFERENCES

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- ⁵ Poster 60: Presented at the 18th international Symposium on Human Identification, 2007 Hollywood.
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