UPHO

TECHNICAL NOTE

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EVALUATION OF UPHO BEAD BEATER ON PLANT TISSUES: AN EFFICIENT AND EASY-TO-USE DISRUPTION DEVICE

The bead beating technology is a mechanical sample disruption method, which originates from the mortar and pestle principle. It involves rapid agitation of samples in vials containing beads with a shaking device, called bead beater. Depending on sample type and bead beater used, a wide range of beads of various size and material (e.g. metal, glass or ceramic) can be used and processing can be performed dry or with buffer at either ambient or cryogenic temperatures.

Initially used to perform lysis of microorganisms, this sample preparation method has since been used for a wide range of applications from tissue disruption to sample homogenization.

This versatility made the use of bead beating very common in biology laboratories, especially when lysis or disruption is performed daily on various sample types (e.g. food industry or biobank). Other advantages of these devices include:

- Processing of multiple samples at once
- Short runtimes
- Efficiency and reproducibility
- Reagent-free procedures and
- Cost effectiveness

THE UPHO SYSTEM

The UPHO system (Figure 1) is a new bead beater from Geneye which enables:

- Short runtime compared to other systems
- Higher shaking frequency (70 Hz) than most systems on the market
- High flexibility for sample size with interchangeable adaptors
- Low laboratory disturbances with a limited noise emission (<65 dB)
- Processing of up to 64 samples simultaneously A

product evaluation run was trialed in an academic laboratory to assess performance for plant tissue preparation against their incumbent system from a major bead-beater provider. In this laboratory, the incumbent system was causing tubes to crack during the grinding process. Risk of system contamination due to tube damage was an important concern and this was included in the evaluation.

REDUCED RISK OF SYSTEM CONTAMINATION

With the incumbent system, large cracks or broken microtubes are typically observed at maximum frequency (25 Hz).

To assess UPHO bead beater on this parameter, 2 mL microtubes were used without samples and containing one 4 mm stainless steel bead. UPHO tubes and tubes typically used in the laboratory were compared for the UPHO system. Tests were performed at 25 Hz shaking frequency.

Results are summarized in Table 1 below.

FREQUENCY	RUN TIME	ADAPTOR PRE-TREATMENT	VESSEL	GRINDING TIME
25 Hz	1:30 min	None	UPHO 2 mL microtube	No cracks
			2 mL microtube from laboratory	No cracks
25 Hz	1:30 min	Liquid nitrogen	UPHO 2 mL microtube	No cracks
			2 mL microtube from laboratory	Slight cracks in the microtube caps

Table 1: Assessment of tube damage caused by bead beating.

When the same frequency is applied, no or lower damage are observed compared to incumbent system. In addition, tubes provided with UPHO system are more robust than the microtubes typically used in the laboratory.

PERFORMANCE

Several protocols were tested to compare the UPHO system with the competitive system for two types of samples (Arabidopsis leaves and rosettes). Adaptors and reaction vials were prefrozen separately in liquid nitrogen.

Two parameters were visually evaluated after processing:

- Aspect of the grinding vessel (20 mL container or 2 mL tubes)
- Powder fineness

Results of the performance study are reported in the Table 2.

SAMPLE	RUN PARAME-TERS	VESSEL SIZE	BEADS	ASPECT OF THE GRINDING VESSEL AFTER PROCESSING	POWER FI-NENESS AFTER PROCESSING (COMPARED TO INCUMBENT)
Arabidopsis leaves (50-100 mg)	1 x 1:30 min at 25 Hz	2 mL	1 x 4 mm stainless steel	Intact	Similar
Arabidopsis rosettes (<1 g)	1 x 1:30 min at 25 Hz	20 mL	1 x 15 mm stainless steel	Intact	Similar
Arabidopsis roset-tes (<1 g)	2 x 1:30 min at 20 Hz	20 mL	1 x 15 mm stainless steel	Intact	Similar
Arabidopsis rosette (0.8 – 2.5 g)	2 x 1:30 min at 25 Hz then 35 Hz	20 mL	6 x 4 mm stainless steel	Intact	Similar
Arabidopsis rosette (0.8 – 2.5 g)	2 x 1:30 min at 35 Hz	20 mL	6 x 4 mm stainless steel	Intact	Finer
Arabidopsis rosette (0.8 – 2.5 g)	1 x 1:30 min at 50 Hz	20 mL	6 x 4 mm stainless steel	Intact	Finer

Table 2: Results after grinding with various protocols on UPHO bead beater.

All samples were pretreated with liquid nitrogen. Comparable results were obtained with *Arabidopsis* root samples.

The powder that was produced with the UPHO device was finely ground (Figure 2) and was of similar quality and even more finely ground than the competitive machine. The samples were still frozen after the removal from the vials.

All protocols applied did not cause any visible damage to the vials. Compared to the incumbent device, higher frequencies could be applied without causing damage to the vials neither.

Different bead beating programs (e.g. 2 cycles of 1:30 min at 35 Hz or 1 cycle of 1:30 min at 50 Hz) can be chosen to produce the same level of power quality. As a result, the protocol can be tailored to the needs of subsequent analysis and time constraint. As an example, the runtime can be divided by 2 when frequency is increased from 35 Hz to 50 Hz.

Figure 2: Arabidopsis samples before and after treatment with UPHO bead beater

CONCLUSION

User feedback highlighted ease-of-use and simplified versatility as major benefits of using the UPHO bead beater. In terms of performance, UPHO bead beater provided similar or finer grinding results for their samples compared to the competitive system. In addition to similar or increased performance, testers experienced:

- Reduced risk of contamination with lower damage to tubes. The frequencies that could be applied without causing damage to the vials were higher than the competitive device.
- Flexibility in terms of protocol design with UPHO higher frequency range. Either runtime can be shortened, or lower shaking frequency can be applied for a smoother processing of sample prior specific subsequent analysis.

The UPHO system was evaluated in other laboratories and has been successfully used to process a wide range of samples including:

- Bacteria and yeast,
- Small animal organs and tissues (e.g. skin, muscle, cartilage, bones),
- Food samples (e.g. tuna, corn, tomato branches, dried olives) and
- Mineral samples (e.g. concrete).

UPHO

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