UPHO

ULTIMATE SAMPLE HOMOGENIZER

CELL DISRUPTION - USER GUIDE

geneye

Cell disruption is an essential step in the workflow to extract and purify important biomolecules, such as nucleic acids and proteins.

When the energy input is **too high**, due to heat generation, cell components (DNA/RNA sequences, proteins) are denatured; if the energy input is **too low**, the cells remain intact, resulting in lower yields.

There are two basic methods for **cell disruption**:
1. Grinding with a suitable buffer at room temperature
2. Grinding with aids such as cryogenic fluids (liquid nitrogen or dry ice)

Beads (working principle is by impact and friction) are commonly used for cell disruption and the overall grinding efficiency depends on the following parameters:

- Size and material of grinding balls
- Type and quantity of sample
- Grinding parameters (run-time, frequency)
- Grinding aids (buffer, cryogenic cooling)

Grinding balls of **3 mm to 7 mm** (metal and ceramic) are typically used for cell disruption. Glass beads with smaller diameters (150 – 600 microns) are used for processing bacteria and yeast.

The disruption of yeasts and bacteria can be very challenging, especially with gram positive/negative or in the form of spores, and may require further steps, such as chemical lysis, to achieve useful results.

With **UPHO** in your laboratory, you will be able to isolate DNA, RNA and proteins from yeasts, microbes and other sample types in a **fast and simple way**.

Cell disruption with UPHO

Preparing consumables:
Generally, before running a program, it is recommended to wash the beads and tubes with ethyl alcohol and sterilize with autoclave.

Sample preparation:
- Cut the sample into small pieces and insert it into a tube (such as the ones provided with the UPHO, or with another laboratory supplier)
- Add deionized water or the desired buffer/solvent solution (see Page #4 for Buffer selection), into the tube, along with the most appropriate bead type;
- Seal the tube and place it into the sample adapter

After a program is finished, centrifuge the tube and its contents, remove the supernatant, recover the beads from the spent tube and wash with ethyl alcohol, dry and store them in a dry environment.

Please note: beads with 0,1-0,5 mm size are difficult to be reused.

The recommended UPHO method parameters not only depend on the sample type the user is working with, but also depends on what experiments will be proceed afterwards.

DNA, RNA and protein extraction

The following tables shows the most common grinding parameters for cell disruption in plastic reaction vials (2 mL).

Sample type	Bead type and quantity	Frequency	Grinding time
Plant tissue	1 x metal or ceramic bead (1 – 6 mm)	60 Hz	40 - 90s
Heart, kidney and muscle	1 x metal or ceramic bead (1 – 6 mm)	65 Hz	60s
Stomach, lung, intestines	1 x metal or ceramic bead (1 – 6 mm)	65 Hz	90 - 120s
Liver, brain, spleen	1 x metal or ceramic bead (1 – 6 mm)	60 Hz	45 - 60s
Skin, fat	2 x metal or ceramic bead (1 – 6 mm)	65 Hz	80 - 90s
Blood vessels (veins, arteries, capillaries)	1 x metal or ceramic bead (1 – 6 mm)	60 Hz	60s
Bacteria	25 - 50 mg glass bead (0,1 mm – 0,5 mm)	60 Hz	60s
Yeast	600 ul glass bead (0,1 mm – 0,5 mm)	60 Hz	60s

Buffer Choice:

The choice of **buffer solution** depends on the grindng protocol and on the sample type the user want to process.

To **extract DNA**, Geneye suggests TPS buffer solution (100 mmol/L Tris- Cl, pH8.0；10 mmol/LEDTA, pH 8.0；1 mol/LKCl) and Trizol for **RNA extraction**: Trizol is a complete, ready-to use reagent, with anti-foaming agents, designed to isolate high quality total RNA (as well as DNA and proteins).

To **extract proteins**, ready-to-use kits can be sourced and used for this procedure.

Small Sample Sizes:

Small volumes (0.5-0.8 mL) of Trizol have been used successfully for small quantities of tissue (1-10 mg) or smaller number of cells (100-10,000). If small volumes are to be processed, Geneye recommends using smaller sizes of tubes, in order to have the tallest possible column of aqueous phase.

The taller the column of liquid, the less likely contamination from the interphase will occur.

Please note:

When the tube has a volume inferior than 2 mL, 5 mm grinding beads could get stuck at the bottom, especially if the tube has a conical shape.

To extract metal elements from the sample, metal beads should not be used in this procedure.

Grinding aids - Liquid nitrogen and dry ice

Hard samples

Hard samples such as: bones, leaf stem, plant roots, hair, etc... and **elastic samples** such as skin, cartilage, connective tissue, blood vessels, etc... can easily be crushed, rather than ground to a powder by applying high mechanical stress, through the impact of beads or pressure and friction from an external source.

When the mechanical grinding force alone is not able to reduce the sample material to a suitable powder for effective extraction, one solution is to use liquid nitrogen (T =-196 °C) or dry ice (T =-78 °C), to freeze the sample and aid grinding by promoting the breaking of such materials into powder.

Heat sensitive samples

In DNA extraction from cells, for example, the DNA is particularly heat sensitive during and after processing. For these applications grinding at low-temperature allows the disruption of groups of cells and cell walls, without denaturing the DNA.

Choice of Grinding Balls and Sample Holders

The choice of ball size and sample holders should be determined based on the sample material. After use, the grinding balls can be discarded or cleaned for re-use, as referred before.

For **grinding with liquid nitrogen**, grinding jars and adapters made from stainless steel or PTFE are available to prepare samples in disposable reaction vials.
They are resistant to the low temperature of liquid nitrogen and no-cross contamination between samples can occur.

Geneye metal adapters are also suitable for this procedure.

The following table represents the suggested grinding settings using liquid nitrogen:

Sample type	Bead type and quantity	Frequency	Grinding time
Plant root, leaf stem	Metal or ceramic bead, 2x 5mm	60 - 65 Hz	15 s
Skin, bone, hair	Metal or ceramic bead, 2x 5mm	60 - 65 Hz	30 s

Please note: For any procedure, please **DO NOT** attempt to open UPHO cover until the grinding process completely stops.

Thank you for choosing UPHO !
We are sure it will become an integral part of your laboratory!