

Guidelines for Cryogenic Grinding of Bone in the Freezer/Mill



SUBJECT (HT001): Grinding and Homogenization

APPARATUS: Freezer/Mill®

APPLICATION: Grinding Bone



Any plant or animal tissue can be ground in a Cole-Parmer Freezer/Mill, and this includes all bone; fresh or aged, wet or dry. Freezer/Mill grinding vials are immersed in liquid nitrogen (LN) before and during grinding, first to make the sample brittle enough to grind, and then to keep it brittle during the actual grinding. The actual grinding is done by a rod-shaped steel impactor that is magnetically shuttled back and forth between two steel end plugs.

Success in Freezer/Mill cryo-grinding depends on two main things:

- The sample must be cold enough to be brittle
- The impactor must be able to move back and forth freely

The quantity of sample per load and the size of the sample pieces can heavily affect the grinding efficiency of the mill, particularly with bone, which can be very tough. A simple test is to load the vial, then shake it back and forth to make sure the impactor can move from end to end of the vial. If the sample pieces are too large or there is too much sample in the vial, the impactor can jam, or not move far enough to grind effectively. In extreme cases, if the impactor is deflected sideways or wedged against the polycarbonate cylinder by a large piece of bone, the cylinder can crack or break.

To grind bone in the standard 6751 vial, we recommend cutting it before grinding into pieces approximately 5 mm in diameter, or smaller. The quantity to be ground in a 6751 Vial can vary from less than half a gram up to four or five grams, depending on the toughness of the sample and the size of the pieces. The optimum sample size and quantity for your samples should be determined empirically. It may be possible to grind one or two large pieces of bone at a time – for example, a 2-gram chunk in one end of the vial – but the uniformity of the results will be better and there will be much less risk of cracking a cylinder if the bone is cut into smaller pieces first.

For bone in the 6751 vial we recommend at least 10 minutes of Pre-Cool time. Depending on the quantity and size of the bone pieces, grinding can take anywhere from 1 minute to 10 minutes. Grinding times longer than 2 minutes should be split into 2- minute grinding periods separated by 2 minutes of cooling. Grinding times can be reduced by increasing the grinding rate from the factory setting of 10 to as much as 14 or 15. If the 6751C polycarbonate center cylinders persist in cracking or breaking, we recommend the use of the 6781S steel vial sets.

The large 6801 vial can grind up to 40 or 50 grams of bone, and the size of the pieces is not important unless they are large enough to prevent the impactor from moving. However, the 6801 vial usually takes longer to grind a sample than the 6751 vial, and for a large sample we recommend 15 minutes of pre-cooling. As with the 6751 vial, grinding should be programmed for two minutes on and two minutes of cooling for as many cycles as necessary to grind the bone to the desired particle size range. The grinding rate can be left at the factory setting of 10 or increased until the impactor starts to “stutter;” the maximum effective rate for the 6801 vial to run smoothly is usually 12.

Grinding performance in the Freezer/Mill is affected by the quantity of sample, the size of the sample pieces, and grinding time. To improve performance, decrease the overall sample quantity, pre-cut the sample into smaller, more uniform pieces, or grind for additional cycles. 10 minutes of pre-cooling for the 6751 vial and 15 minutes of pre-cooling for the 6801 vial ought to be enough for bone, but for very large pieces of bone in the 6801 you may need to increase the pre-cooling time to 20 minutes.

Again, we recommend running the Freezer/Mill for 2 minutes per grinding cycle with 2 minutes of cooling between cycles, to let the sample and coil cool back down. It is possible to run the Freezer/Mill continuously, but as the coil heats up it becomes less efficient. Bone will probably remain “grindable” for many minutes of continuous grinding, but for difficult-to-grind samples like synthetic fibers and polymer sheets, it is probably better to keep them as cold as possible during grinding.

Generally speaking, all components of Freezer/Mill vials can be cleaned with hot water and detergent or soap, and decontaminated by dipping in a solution of 10% bleach or dilute mineral acids. After cleaning they should be dried. Polycarbonate cylinders can be damaged by excessive heat (as in autoclaving) or exposure to organic solvents including alcohol. Steel impactors and end plugs, and steel cylinders, may be sterilized by disinfectants, alcohol, autoclaving, etc., but should not be left in contact with water and aqueous liquids for any length of time, as magnetic steel can rust under these conditions.