BEAD BEATING

INTRODUCTION & GUIDELINES

Bead beating is an effective process used to disrupt a wide range of biological samples. It is achieved by rapidly agitating samples with grinding media (balls or beads) in a bead beater. Samples can be processed with or without buffer or solvent at room temperature or cryogenically.

The Geno/Grinder® and MiniG® are high throughput homogenizers and can process samples in deep well plates as well as other formats (up to 50ml tubes), and have a linear motion that focuses the kinetic energy of the grinding media on the sample instead of on the sides of the container. Consequently, even resilient samples such as seeds are most effectively homogenized by these linear motion bead beaters.

Sample variability

Different samples vary in their resiliency to homogenization by bead beating. Even moderately resilient samples can be effectively homogenised using an apparatus such as the Geno/Grinder and MiniG.

An effective approach to homogenization is to find a balance between sample mass, vessel volume and grinding media material and size. A method that is effective with one sample will not necessarily be effective on another, so as is often the case some method development will be necessary to establish the optimum protocol to reach the desired endpoint.

Processing Parameters

Whatever the format, sample vessels must be able to withstand the impact of grinding beads and balls. Polycarbonate vessels are an excellent choice because they are clear, durable and impact resistant and have the ability to withstand cryogenic temperatures. However because PC is incompatible with many organic solvents durable polyethylene or polypropylene tubes and deep well plates can be used instead – although these are susceptible to mechanical breakage when used with metal grinding balls.

The size and type of sample must be matched with a suitably large homogenisation container and grinding media that together will efficiently homogenise the sample. Grinding media must move freely to impact the sample thus tubes must not be overfilled with beads or buffer. As a general rule sample should not take up more than 1/6 of the volume of the vessel; and the grinding media no more than 1/3.

Grinding media can be categorised as:

Beads – a pool of beads falling within a size range. Beads with small diameters are best for disrupting smaller samples such as bacteria or yeast, will larger beads or grinding balls are best for homogenising plant and animal tissues.

Balls – spherical and precision ground to a specific diameter. (Where stainless steel reacts with phenolic compounds in some buffers, ceramic satellites can be used as an alternative).

Ceramic satellites/grinding resins – sharp and irregularly shaped composites.

High density grinding media such as stainless steel and zirconia are usually more effective than silica beads; however the former materials tend to generate heat during grinding. In addition some material sheared from grinding beads can inhibit some enzyme reactions. Consequently, there is no one media useful for all applications.

Generally grinding media should be cleaned to decontaminate before using. Acid washing is the most common method although heat treating removes a potential source of nuclease or nucleic acid. Also low binding beads are available that reduce non-specific binding thereby allowing more analyte to remain in suspension.

Cryogenic Homogenisation

Some hard to grind samples will need to be ground cryogenically. Both Geno/Grinder & MiniG have a range of Kryo-Tech® accessories that allow chilled samples to be placed in and homogenised whilst mounted in a chilled Cryo-Block. This technique is useful for both hard to grind samples and where temperature sensitive samples need to be prepared.

SAMPLE HOMOGENIZATION

Micro-Organisms

Bacteria

The small size of bacteria and their concentration in the sample are important variables when bead beating. 100µm Zirconia or silica beads are most effective and analyte losses can be minimised by using low binding beads. Samples can be processed in multi-well plates, 0.5ml. 2ml, 4ml or 15ml vials. A high beating speed is recommended.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15 ml Vials
Buffer volume	600µl	600µl	1.5ml	6ml
Bead volume (100µm beads)	400µl	300µl	0.8ml	3ml
Processing time	2-5 mins	5 mins	5 mins	5-10 mins
Speed	High	High	High	High

SUGGESTED PRODUCTS:

100µm grinding beads

#2168 - Silica, low binding grinding beads

#2181- Zirconium low binding grinding beads

100µm Pre-filled 2ml vials

#2301-100MB - 100µm Silica beads, MB grade

#2302-100AW - 100µm acid washed Zirconia beads


2168 Silica Grinding Beads


2301-100MB Pre-filled 2 ml vial

Complex Matrices – Soil, Feces, Biofilms

Air, water, soil, sediments, feces and surfaces supporting biofilm can all be complex with a mixture of different particle sizes and composition. Depending on the composition and density of the biotic element these samples are often concentrated by centrifugation or filtration.

Complex samples usually require a mixture of different grinding media to effectively disrupt both organisms and particles present. Larger beads will break up soil particles, and small beads are effective for disrupting micro-organisms such as bacteria & fungi. Samples can be processed in a range of vials.

MATRIX	SAMPLE	BEAD MIXTURE
Membrane filter	¼ section of 47mm filter in 2ml tube	100mg each of 100 & 400 µm beads
Soil/sediment/feces	250mg in 2ml disruption tube	100mg of 100µm silica; 500mg of 400µm beads; and 2 x 4mm glass beads
Biofilm	Substrate with biofilm fits into disruption vial (objective is to remove the film from the substrate)	1/6 volume with 1mm Zirconia beads initially. Then smaller beads if required

SUGGESTED PRODUCTS FOR COMPLEX MATRICES:

Beads – #2181 - 100µm Zirconia; #2168 - 100µm Silica; #2167 - 400µm Silica

Prefilled 2ml Tubes – #2303-MM3 - Mixed 100µm, 1.4mm Zirconia & 4mm silica beads, #2302-1000AW - 1.0mm Zirconia beads


2168 Silica Grinding Beads


2303-MM3 Pre-filled 2 ml vial

Yeast

Small yeast cells such as Pichia are best homogenised with 200µm Zirconia beads. Larger yeast cells such as Saccharomyces are best disrupted using 400µm silica or Zirconia beads. If cell densities are low it is advantageous to use low binding media. Samples can be disrupted in a range of vials.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15 ml Vials
BUFFER VOLUME	600µl	600µl	1.5ml	6ml
BEAD VOLUME (400µM BEADS)	400µl	300µl	0.8ml	3ml
PROCESSING TIME	2-5 mins	5 mins	5-10 mins	5-10 mins
SPEED	High	High	High	High

SUGGESTED PRODUCTS:

Beads

- #2182, 200µm Zirconia for Pichia
- #2167, 400µm Silica for Saccharomyces

Pre-filled 2ml vials

- #2304-400, 400µm Silica Beads
- #2302-200AW, 200µm Zirconium Beads

2182 Silica Grinding Beads

2304-400 Pre-filled 2 ml vial

Fungi

Fungi, in all its polymorphic forms, can be homogenised with several types of beads. Loosely packed mycelia are effectively disrupted with 800µm or 1.7mm Zirconia or silica beads. However as the density of the mycelium increases as with fruiting bodies, larger beads and small grinding balls are needed to tear the thallus and disrupt the cells. Solid fruiting bodies can be disrupted with 2.8mm steel balls. Some fruiting bodies and complex structures such as lichens may require a mix of sizes, much like environmental samples. Low binding beads minimises loss of analytes. Samples can be processed in a range tubes or vials.

SAMPLE TYPE	Features	Disruption tube, 2ml vials	Deep Well Plate (Square wells)	4ml Vials	15ml Vials
MYCELIUM	Pellet Size	50µl	50µl	500mg	Up to 2g
	Bead Size	800µm Zr	800µm Zr	800µm Zr	800µm Zr
	Bead Mass	600mg	600mg	1200mg	3g
PELLICLE	Wet weight	200mg	200mg	500mg	Up tp 2g
	Ball size	1.7mm Zr	1.7mm Zr	1.7mm Zr	1.7mm Zr
	Ball mass	570mg	570mg	1200mg	3g
THALLUS	Wet Weight	200mg	200mg	500mg	Up to 2g
	Ball size	2.8mm SS	2.8mm SS	2.8mm SS	2.8mm SS
	No of balls	8	8	15	30
		Processing time: 2-5 mins	Processing time: 2-5 mins	Processing time: 5 mins	Processing time: 5-10 mins
		Buffer Volume: 600µl	Buffer Volume: 600µl	Buffer Volume: 1.5ml	Buffer Volume: 6ml
		Speed: High	Speed: High	Speed: High	Speed: High

SUGGESTED PRODUCTS:

Grinding Beads – #2305-28 - 2.8mm stainless steel balls for thallus (eg Mushroom)

Pre-filled 2ml tubes
#2302-1700AW - 1.7mm Zirconia beads for pellicle;
#2305-2800SS - 2.8mm stainless steel balls or
#2302-6000AW - 6.0mm Zirconia beads for thallus

#2305-28 - 2.8mm stainless steel balls

#2302-1700 AW Pre-filled 2 ml vial

PLANT SAMPLES

Leaf Tissue

Samples are often prepared using leaf punches. Small samples of up to 50mg for genetic extraction can be processed in deep well plates using 4mm (or similar sized) stainless steel or Zirconia balls. Denser leaf tissue may require larger beads and balls. Samples over 100mg are best processed in appropriately sized grinding vials. Up to 200mg is best done in 4ml PC vials with one 8 or 9.5mm ball. Up to 1g use 15ml PC tube with 2 x 11mm balls. Use Zirconia balls if stainless steel reacts with corrosive buffers. Bead beating in solution can be used to generate small fragments of DNA for processes such as PCR. Cryo-Blocks may be used to keep samples frozen and dissipate heat during processing.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15ml Vials
Sample Mass	50mg	50mg	200mg	Up to 1g
Buffer volume	600µl	500µl	2ml	6ml
Grinding Balls	8 x 2.8mm	1 x 4mm	1 x 9.5mm	2 x 11mm
Processing time	1 min	2-5 mins	2 mins	2-5 mins
Speed	2/3	2/3	2/3	2/3

SUGGESTED PRODUCTS:

Grinding Beads – #2305-28, 2.8mm stainless steel balls; 2150, 4mm stainless steel balls; 2155, 9.5mm stainless steel balls; 2156, 11mm stainless steel balls

Pre-filled 2ml tubes – #2302-3000AW, 3mm Zirconia; #2305-2800SS, 2.8mm stainless steel balls.


#2305-28 - 2.8mm stainless steel balls


#2305-2800SS Pre-filled 2 ml vial

Plant stems

Stems have a high concentration of vascular tissue which is resistant to shearing and grinding. Small, young stems can be treated like leaves – less than 50mg in deep well plates with one 4mm grinding ball. Tougher larger stems are best processed in 4 or 15ml grinding vials. Up to 200mg samples, use 4ml vials with one 8 or 9.5mm ball; up to one gram use the 15ml PC vial with 2 x 11mm steel balls.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15ml Vials
Sample Mass	10mg	10mg	Up to 25mg	Up to 100mg
Buffer volume	500µl	500µl	1ml	5ml
Bead volume	400µl	300µl	1ml	3ml
Processing time	2-5 mins	2-5 mins	5 mins	5-10 mins
Speed	High	High	High	High

SUGGESTED PRODUCTS:

Grinding Beads – #2305-28 - 2.8mm stainless steel ball; #2150 - 4mm stainless steel balls; #2155 - 9.5mm stainless steel balls; #2156 -11mm stainless steel balls.

Pre-filled 2ml tubes – #2240-PC 24 x 5ml polycarbonate vials preloaded with 9.5mm stainless steel ball; #2250, pre-cleaned polycarbonate, short form 15ml vial set, preloaded a 11mm stainless steel ball.


#2305-28 - 2.8mm stainless steel balls


##2240-PC - Pre-filled 2 ml vial

Pollen

Pollen has a resilient wall and requires more dense Zirconia beads for homogenization. As the size pollen is a very broad, bead size must be matched to the type of pollen. Beads that are too small will not crack open pollen, whilst using large beads may result in too few collisions. Pollen less than 10µm should be disrupted with 200µm Zirconia beads; 10-50µm pollen needs 400µm Zirconia beads; and >50µm pollen is most effectively homogenised using 800µm beads. A variety of tubes and vials can be used.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15ml Vials
Sample Mass	10mg	10mg	Up to 25mg	Up to 100mg
Buffer volume	500µl	500µl	1ml	5ml
Bead volume	400µl	300µl	1ml	3ml
Processing time	2-5 mins	2-5 mins	5 mins	5-10 mins
Speed	High	High	High	High

SUGGESTED PRODUCTS:

Grinding Beads – #2181, 100µm Zirconia; 400µm Zirconia; 800µm Zirconia

Pre-filled 2ml tubes – #2302-100AW2, 100µm Zirconia; 400µm Zirconia; 800µm Zirconia


#2305-28 - 2.8mm stainless steel balls


#2302-100AW2 Pre-filled 2 ml vial

Seeds

These samples are effectively homogenised by dry grinding with a disproportionately large ball to crack these hard samples. Freeze drying or air dried seeds with sufficiently low water content may allow grinding to a fine powder.

The composition of grinding vials and tubes is important as plastic tends to heat and soften during grinding. However polycarbonate will ensure a hard grinding surface during homogenisation. Most seeds need a minimum 9.5mm ball within the polycarbonate vial. Single seeds are commonly processed in a 4ml vial, but multiple seeds require a 15ml PC vial. If stainless steel balls do not have enough energy to grind seeds substitute with tungsten carbide balls. Some seeds such as palm nuts may require a Freezer/Mill® for truly effective grinding.

	4ml Vials	15ml Vials
Sample Mass	One seed	Up to 5g
Grinding Balls	1 x 9.5mm	2 x 11mm
Processing time	2-3 mins	3-5 mins
Speed	High	High

SUGGESTED PRODUCTS:

Grinding Beads: #2155, 9.5mm stainless steel balls; #2156, 11mm stainless steel balls.

Grinding Vial Sets – #2240-PC – 24 x 5ml polycarbonate vials preloaded with 9.5mm stainless steel ball; #2250, pre-cleaned polycarbonate, short form 15ml vial set, preloaded a 11mm stainless steel ball.


#2155 - 4mm stainless steel balls


#2250 - 15mL polycarbonate vial

Soft Animal Tissue, Liver/Brain

Soft animal tissues are easily homogenised by bead beating either with buffer or cryogenically – the latter to recover high molecular weight DNA. Samples <100mg can be processed in tubes with 1.4 – 3.0mm Zirconia beads. Also deep well plates with 4mm or 6mm satellite would be effective.

Larger samples up to 200mg are best homogenised in grinding vials with 8 or 9.5mm balls. Up to 2 grams of animal tissue can be homogenised in a 15ml tube with 11mm balls. Polyethylene vials and Zirconia balls should be used if phenol based buffers act aggressively on other materials.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15ml Vials
Sample Mass	20mg	20mg	100-200mg	Up to 2mg
Buffer volume	600µl	200µl	1ml	6ml
Grinding balls	8 x 2.8mm	1 x 4mm	1 x 9.5mm	2 x 11mm
Processing time	2 mins	2 mins	2-3 mins	3-5 mins
Speed	High	High	High	High

SUGGESTED PRODUCTS:

Grinding Beads:
#2305-28, 2.8mm stainless steel ball;
#2150, 4mm stainless steel balls;
#2155, 9.5mm stainless steel balls;
#2156, 11mm stainless steel balls

Grinding Vial Sets:
#2240-PC, 24 x 5ml polycarbonate vials each loaded with 9.5mm stainless steel ball;

#2250,pre-cleaned polycarbonate, short form 15ml vial set, each preloaded with a 11mm stainless steel ball.

Pre-filled 2ml tubes:
#2305-2800SS, 2.8mm stainless steel balls


#2155 - 4mm stainless steel balls


#2250 - 15mL Polycarbonate Vial

Fibrous Animal Tissue, Muscle/Heart/Lung

These samples contain significant amounts of connective tissue and microfilaments so need considerably more force to homogenise than softer tissues. Fibrous tissues can be homogenised in buffer or cryogenically. For samples less than 50mg use disruption tubes with 1.7 – 3.0mm Zirconia beads or small SS balls. Alternatively use deep well plates with 4 or 6mm balls. Larger samples should be homogenised in grinding vials. Up to 200mg in 4ml vials with one 9 mm ball; and up to 2 grams in 15ml tubes with 2 x 11mm balls. Polyethylene tubes should be used where organic solvents will react with polycarbonate. Zirconia oxide satellites are resistant to corrosive chemicals and can be used with garnet shards to rip and cut the tissue.

	Disruption tubes, 2ml	Deep Well Plates (Square wells)	4ml Vials	15ml Vials
Sample Mass	20mg	20mg	100-200mg	Up to 2mg
Buffer volume	600µl	200µl	1ml	6ml
Grinding balls	8 x 2.8mm	1 x 4mm	1 x 9.5mm	2 x 11mm
Processing time	2 mins	2 mins	3-5 mins	5-10 mins
Speed	High	High	High	High

SUGGESTED PRODUCTS:

Grinding Beads – #2305-28, 2.8mm stainless steel ball; #2150, 4mm stainless steel balls; #2155, 9.5mm stainless steel balls; #2156, 11mm stainless steel balls.

Pre-filled tubes - #2305-2800SS, 2.8mm stainless steel balls or #2302-6000AW, 6.0mm Zirconia beads.

Grinding Vials – #2240-PC – 24 x 5ml polycarbonate vials each loaded with 9.5mm stainless steel ball;

#2250,pre-cleaned polycarbonate, short form 15ml vial set, each preloaded with a 11mm stainless steel ball.

(Consider Cryogenic options for 4ml & 15ml tubes)


#2150 - 4mm stainless steel balls


#2302-6000AW Pre-filled 2 ml vial

Elastic Animal Tissue, Skin, Sclera/Cartilage

Some tissues with larger amounts of collagen can be difficult to homogenise. In order to homogenise effectively the mass of the sample must be relatively small compared to the grinding media and vessel. Samples <20 mg are best homogenised in 4ml PC vials with one 9.5mm SS grinding ball. Larger samples are best homogenised in a 15ml PC vial with 2 x 11mm SS balls. It is often necessary to homogenise elastic samples cryogenically.

	4ml Vials	15ml Vials
Sample Mass	20mg	Up to 250g
Grinding Balls	1 x 9.5mm	2 x 11mm
Processing time	5 mins	5-10 mins
Speed	High	High

SUGGESTED PRODUCTS:

Grinding Beads – #2155, 9.5mm stainless steel balls; #2156, 11mm stainless steel balls.

Grinding Vials – #2240-PC – 24 x 5ml polycarbonate vials each loaded with 9.5mm stainless steel ball; #2250,pre-cleaned polycarbonate, short form 15ml vial set, each preloaded with a 11mm stainless steel ball.

(Consider Cryogenic options for 4ml & 15ml tubes)


#2155 - 9.5mm stainless steel balls


#2240-PC - 5 mL Polycarbonate Vial

Resilient Animal Tissue, Bone, Hair/Nail

These samples are best homogenised using a disproportionately large grinding ball to crack the sample. Often they need to be ground cryogenically to make the samples extra brittle. Often, using the Freezer/Mill is more effective for these types of sample. Due to the nature of these samples a hard plastic such as PC must be used. Samples less than 100mg are best homogenised in 4ml vials with one 9.5mm SS or tungsten carbide ball. Larger samples should be processed in a 15ml PC vial with 2 x 11mm SS or TC balls.

	4ml Vials	15ml Vials
Sample Mass	100mg	Up to 250g
Buffer Volume	1ml	6ml
Grinding Balls	1 x 9.5mm	2 x 11mm
Processing time	5 mins	5-10 mins
Speed	High	High

SUGGESTED PRODUCTS:

Grinding Beads – #2155, 9.5mm stainless steel balls; #2156, 11mm stainless steel balls.

Grinding Vials – #2240-PC – 24 x 5ml polycarbonate vials each loaded with 9.5mm stainless steel ball; #2250,pre-cleaned polycarbonate, short form 15ml vial set, each preloaded with a 11mm stainless steel ball.

(Consider Cryogenic options for 4ml & 15ml tubes)


#2155 - 9.5mm stainless steel balls


#2240-PC - 5 mL Polycarbonate Vial

CONCLUSION

The Geno/Grinder & MiniG are proven and effective high throughput tissue homogenisers. Extremely versatile and with the capacity to process multiple sample simultaneously. The linear action has been shown to be highly effective at lysing cells and disrupting tissues for applications from removal of genetic material, protein isolation and extraction of low level residues such as pesticides.

As in all types of analytical process, good and consistent sample preparation is essential to ensure high quality analytical results. The notes above illustrate some of the issues that need to be considered, as well as providing a good starting point when working with various sample types.

However to optimise the procedure for particular samples some further method development will be needed. Once this has been established the method can be used repeatedly for consistent sample preparation leading to reliable analysis.

If you need more guidance or advice please contact your SPEX® SamplePrep representative or visit www.spexsampleprep.com

Appendices:

Properties of Grinding Media:

	Silica	Zirconia Silicate	Zirconia Oxide 1	Zirconia Oxide 2	Stainless Steel
Density (g/cc)	2.25	3.84	6.0	6.2	7.9
Durability	Low	Medium	High	High	Medium
Relative hardness	+	++	++++	++++	++

These guidelines are based on an original document written by Lindsay E. Gibbons, Halley C.G. Brangs, and David W. Burden, OPS Diagnostics. SPEX SamplePrep LLC wishes to thank Dave Burden for his assistance and permission to re-publish the document.

SPEX SamplePrep
65 Liberty Street, Metuchen, NJ 08840 USA
Tel: 732-623-0465
Eamil: learnmore@spex.com