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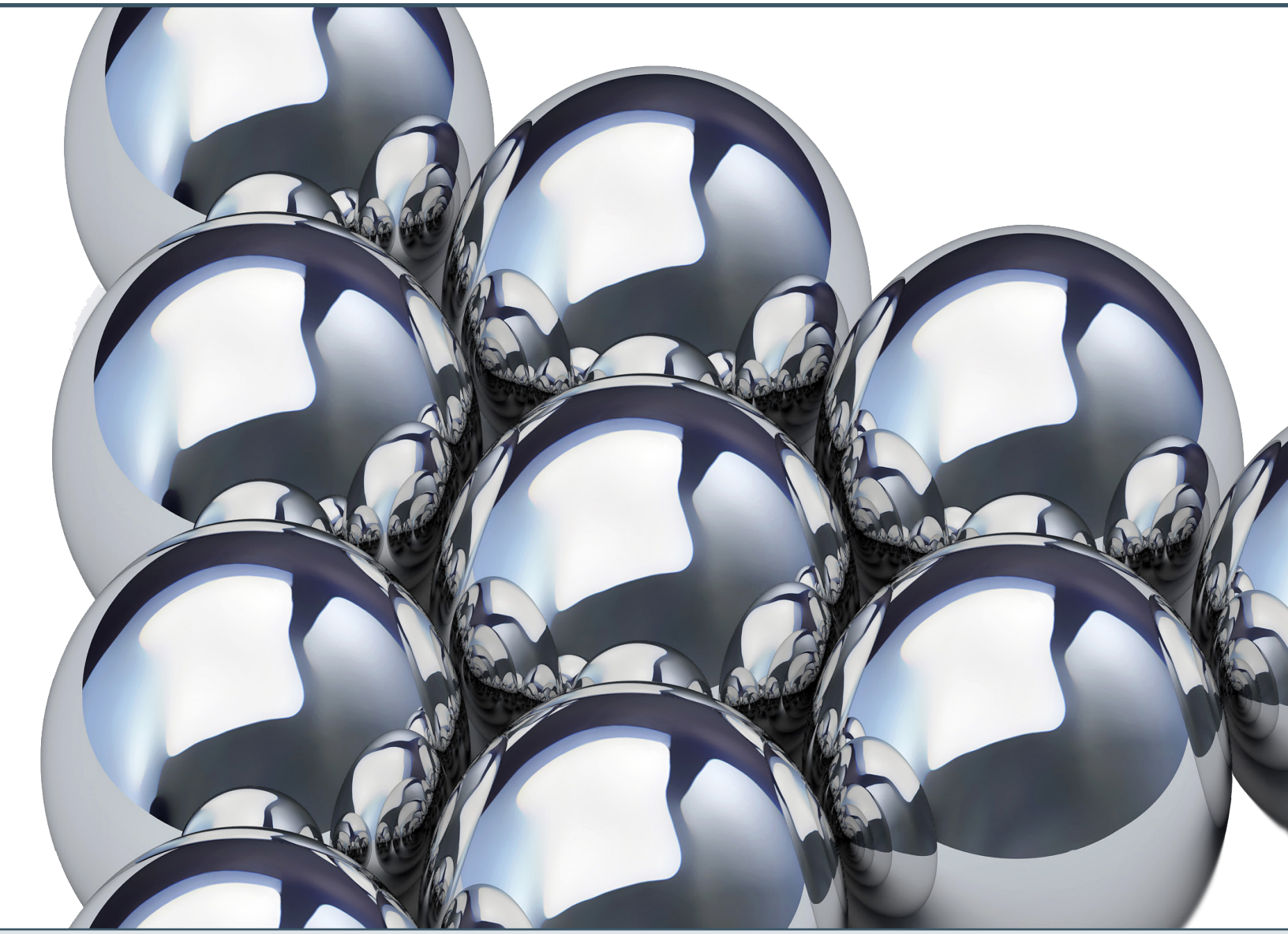
# Bead Beating

## Introduction and Guidelines



**APPARATUS:** Geno/Grinder®

**APPLICATION:** Bead Beating

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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 that can process samples in deep well plates as well as other formats (up to 50 mL tubes). The homogenizers 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 homogenized 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, and material and size. A method that is effective with one sample will not necessarily be effective on another. 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 (PC) 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 (PE) or polypropylene (PP) 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 homogenization container and grinding media that together will efficiently homogenize 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, the sample should not take up more than one sixth of the vessel's volume and the grinding media no more than one third of the vessel's volume.

**Grinding Media Characterizations:**

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, while larger beads or grinding balls are best for homogenizing 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 Homogenization**

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

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# SAMPLE HOMOGENIZATION

## Microorganisms

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

| | Disruption tubes, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Buffer volume | 600 µL | 600 µL | 1.5 mL | 6 mL |
| Bead volume (100 µm beads) | 400 µL | 300 µL | 0.8 mL | 3 mL |
| Processing time | 2–5 mins | 5 mins | 5 mins | 5–10 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2168 – silica, low-binding grinding beads

#2181 – zirconia low-binding grinding beads

Pre-filled Vials

#2301-100MB – 100 µm silica beads, molecular biology grade

#2302-100AW – 100 µm 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 microorganisms such as bacteria and fungi. Samples can be processed in a range of vials.

| Matrix | Sample | Bead mixture |
|---|---|---|
| Membrane filter | ¼ section of 47 mm filter in 2 mL tube | 100 mg each of 100 and 400 µm beads |
| Soil/sediment/feces | 250 mg in 2 mL disruption tube | 100 mg of 100 µm silica; 500 mg of 400 µm beads; 2 x 4 mm glass beads |
| Biofilm | Substrate with biofilm fits into disruption vial (objective is to remove the film from the substrate) | ⅙ volume with 1 mm zirconia beads initially, then smaller beads if required |

**Suggested Products**

Grinding Beads

#2181 – 100 µm zirconia

#2168 – 100 µm silica

#2167 – 400 µm silica

Pre-filled Vials

#2303-MM3 – Mixed 100 µm, 1.4 mm zirconia and 4 mm silica beads

#2302-1000AW – 1.0 mm zirconia beads

#2168 Silica Grinding Beads

#2303-MM3 Pre-filled 2 mL Vial

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## Yeast

Small yeast cells such as Pichia are best homogenized with 200 µm zirconia beads. Larger yeast cells such as Saccharomyces are best disrupted using 400 µm 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, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Buffer volume | 600 µL | 600 µL | 1.5 mL | 6 mL |
| Bead volume (400 µm beads) | 400 µL | 300 µL | 0.8 mL | 3 mL |
| Processing time | 2–5 mins | 5 mins | 5–10 mins | 5–10 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2182 – 200 µm zirconia for Pichia

#2167 – 400 µm silica for Saccharomyces

Pre-filled Vials

#2302-200AW – 200 µm zirconia beads

#2304-400 – 400 µm silica beads

#2182 Silica Grinding Beads

#2304-400 Pre-filled 2 mL Vial

## Fungi

Fungi, in all its polymorphic forms, can be homogenized with several types of beads. Loosely packed mycelia are effectively disrupted with 800 µm or 1.7 mm 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 2.8 mm stainless steel balls. Some fruiting bodies and complex structures such as lichens may require a mix of sizes, much like environmental samples. Low-binding beads minimize loss of analytes. Samples can be processed in a range of tubes or vials.

| Sample type | Features | Disruption tubes, 2 mL vials | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|---|
| Mycelium | Pellet size | 50 µL | 50 µL | Up to 10 mL | Up to 50 mL |
| | Zr Bead size | 800 µg | 800 µg | 800 µg | 800 µg |
| | Bead mass | 600 mg | 600 mg | 1200 mg | 3 g |
| Pellicle | Wet weight | 200 mg | 200 mg | 500 mg | Up to 2 g |
| | Zr Bead size | 1.7 mm | 1.7 mm | 1.7 mm | 1.7 mm |
| | Bead mass | 570 mg | 570 mg | 1200 mg | 3 g |
| Thallus | Wet weight | 200 mg | 200 mg | 500 mg | Up to 2 g |
| | SS Ball size | 2.8 mm | 2.8 mm | 2.8 mm | 2.8 mm |
| | Number of balls | 8 | 8 | 15 | 30 |
| | Processing time | 2–5 mins | 2–5 mins | 5 mins | 5–10 mins |
| | Buffer volume | 600 µL | 600 µL | 1.5 mL | 6 mL |
| | Speed | High | High | High | High |

#2305-28 2.8 mm Stainless Steel Balls

#2302-1700AW Pre-filled 2 mL Vial

**Suggested Products**

Grinding Beads — #2305-28 – 2.8 mm stainless steel balls for thallus (e.g. mushrooms)

Pre-filled Vials — #2302-1700AW – 1.7 mm zirconia beads for pellicle; #2305-2800SS – 2.8 mm stainless steel balls
#2302-6000AW – 6.0 mm zirconia beads for thallus

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# PLANT SAMPLES

## Leaf Tissue

Samples are often prepared using leaf punches. Small samples of up to 50 mg for genetic extraction can be processed in deep well plates using 4 mm (or similar sized) stainless steel or zirconia balls. Denser leaf tissue may require larger beads and balls. Samples over 100 mg are best processed in appropriately sized grinding vials. Up to 200 mg is best done in 4ml PC vials with one 8 or 9.5 mm ball. Up to 1 g use 15 mL PC tube with 2 x 11 mm 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. Cryoblocks may be used to keep samples frozen and dissipate heat during processing.

| | Disruption tubes, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Sample mass | 50 mg | 50 mg | 200 mg | Up to 1 g |
| Buffer volume | 600 µL | 500 µL | 2 mL | 6 mL |
| Grinding balls | 8 x 2.8 mm | 1 x 4 mm | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 1 min | 2–5 mins | 2 mins | 2–5 mins |
| Speed | Medium | Medium | Medium | Medium |

**Suggested Products**

Grinding Beads

#2305-28 – 2.8 mm stainless steel balls

#2150 – 4 mm stainless steel balls

#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Pre-filled Vials

#2302-3000AW – 3 mm zirconia

#2305-2800SS – 2.8 mm stainless steel balls

#2305-28
2.8 mm 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 50 mg in deep well plates with one 4 mm grinding ball. Tougher larger stems are best processed in 4 or 15 mL grinding vials. Up to 200 mg samples, use 4 mL vials with one 8 or 9.5 mm ball; up to one gram, use the 15 mL PC vial with 2 x 11 mm stainless steel balls.

| | Disruption tubes, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Sample mass | 10 mg | 10 mg | Up to 25 mg | Up to 100 mg |
| Buffer volume | 500 µL | 500 µL | 1 mL | 5 mL |
| Bead volume | 400 µL | 300 µL | 1 mL | 3 mL |
| Processing time | 2–5 mins | 2–5 mins | 5 mins | 5–10 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2305-28 – 2.8 mm stainless steel ball

#2150 – 4 mm stainless steel balls

#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Pre-filled Vials

#2240-PC – 24 x 5 mL polycarbonate vials preloaded with 9.5 mm stainless steel ball

#2250 – pre-cleaned polycarbonate, short form 15 mL vial set, preloaded a 11 mm stainless steel ball

#2305-28
2.8 mm Stainless Steel Balls

#2240-PC
Pre-filled
2 mL Vial

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## Pollen

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

| | Disruption tubes, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Sample mass | 10 mg | 10 mg | Up to 25 mg | Up to 100 mg |
| Buffer volume | 500 µL | 500 µL | 1 mL | 5 mL |
| Bead volume | 400 µL | 300 µL | 1 mL | 3 mL |
| Processing time | 2–5 mins | 2–5 mins | 5 mins | 5–10 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2181 – zirconia low-binding grinding beads

Pre-filled Vials

#2302-100AW2 – 100 µm zirconia; 400 µm zirconia; 800 µm zirconia

#2181 Grinding Beads

#2302-100AW2 Pre-filled 2 mL Vial

## Seeds

These hard samples are effectively homogenized by dry grinding with a disproportionately large ball to crack them. 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 because plastic tends to heat and soften during grinding. However, PC will ensure a hard grinding surface during homogenization. Most seeds need a minimum 9.5 mm ball within the PC vial. Single seeds are commonly processed in a 4 mL vial, but multiple seeds require a 15 mL 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.

| | 4 mL vials | 15 mL vials |
|---|---|---|
| Sample mass | One seed | Up to 5 g |
| Grinding balls | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 2–3 mins | 3–5 mins |
| Speed | High | High |

**Suggested Products**

Grinding Beads

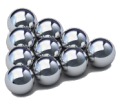
#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Pre-filled Vials

#2240-PC – 24 x 5 mL polycarbonate vials pre-loaded with a 9.5 mm stainless steel ball

#2250 – pre-cleaned polycarbonate, short form 15 mL vial set



#2155 9.5 mm Stainless Steel Balls

#2250 Polycarbonate Vial, 15 mL

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# ANIMAL TISSUE

## Soft Animal Tissue – Liver/Brain

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

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

| | Disruption tubes, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Sample mass | 20 mg | 20 mg | 100–200 mg | Up to 2 g |
| Buffer volume | 600 µL | 200 µL | 1 mL | 6 mL |
| Grinding balls | 8 x 2.8 mm | 1 x 4 mm | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 2 mins | 2 mins | 2–3 mins | 3–5 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2305-28 – 2.8 mm stainless steel ball

#2150 – 4 mm stainless steel balls

#2155 – 9.5 mm stainless steel balls;

#2156 – 11 mm stainless steel balls

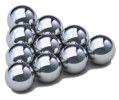
Grinding Vial Sets

#2240-PC – 24 x 5 mL polycarbonate vials each loaded with one 9.5 mm stainless steel ball

#2250 – pre-cleaned polycarbonate, short form 15 mL vial set, each preloaded with one 11 mm stainless steel ball

Pre-filled Vials

#2305-2800SS – 2.8 mm stainless steel balls



#2150
4 mm Stainless Steel Balls

#2250
Polycarbonate Vial, 15 mL

## Elastic Animal Tissue – Skin/Sclera/Cartilage

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

| | 4 mL vials | 15 mL vials |
|---|---|---|
| Sample mass | 20 mg | Up to 250 mg |
| Grinding balls | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 5 mins | 5–10 mins |
| Speed | High | High |

**Suggested Products**

Grinding Beads

#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Grinding Vials

#2240-PC – 24 x 5 mL polycarbonate vials each loaded with 9.5 mm stainless steel ball

#2250 – pre-cleaned polycarbonate, short form 15 mL vial set, each preloaded with two 11 mm stainless steel balls

(Consider cryogenic options for 4 mL & 15 mL tubes)

#2155
9.5 mm Stainless Steel Balls

#2240-PC
Polycarbonate Vial, 5 mL

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## Fibrous Animal Tissue – Muscle/Heart/Lung

These samples contain significant amounts of connective tissue and microfilaments so need considerably more force to homogenize than softer tissues. Fibrous tissues can be homogenized in buffer or cryogenically. For samples less than 50 mg use disruption tubes with 1.7 to 3.0 mm zirconia beads or small stainless steel balls. Alternatively use deep well plates with 4 or 6 mm balls. Larger samples should be homogenized in grinding vials. Up to 200 mg in 4 mL vials with one 9 mm ball; and up to 2 grams in 15 mL tubes with 2 x 11 mm 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, 2 mL | Deep well plates (square wells) | 4 mL vials | 15 mL vials |
|---|---|---|---|---|
| Sample mass | 20 mg | 20 mg | 100–200 mg | Up to 2 g |
| Buffer volume | 600 µL | 200 µL | 1 mL | 6 mL |
| Grinding balls | 8 x 2.8 mm | 1 x 4 mm | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 2 mins | 2 mins | 3–5 mins | 5–10 mins |
| Speed | High | High | High | High |

**Suggested Products**

Grinding Beads

#2305-28 – 2.8 mm stainless steel ball

#2150 – 4mm stainless steel balls

#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Grinding Vials

#2240-PC – 24 x 5 mL polycarbonate vials each loaded with one 9.5 mm stainless steel ball

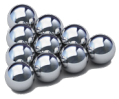
#2250– pre-cleaned polycarbonate, short form 15 mL vial set, each preloaded with one 11 mm stainless steel ball

Note: Consider cryogenic options for 4 mL & 15 mL tubes.

Pre-filled Tubes

#2305-2800SS – 2.8mm stainless steel balls or

#2302-6000AW – 6.0 mm zirconia beads



#2150
4 mm Stainless Steel Balls

2302-6000AW
Pre-filled
2 mL Vial

## Resilient Animal Tissue – Bone/Hair/Nail

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

| | 4 mL vials | 15 mL vials |
|---|---|---|
| Sample mass | 100 mg | Up to 250 g |
| Buffer volume | 1 mL | 6 mL |
| Grinding balls | 1 x 9.5 mm | 2 x 11 mm |
| Processing time | 5 mins | 5–10 mins |
| Speed | High | High |

**Suggested Products**

Grinding Beads

#2155 – 9.5 mm stainless steel balls

#2156 – 11 mm stainless steel balls

Grinding Vials

#2240-PC – 24 x 5 mL polycarbonate vials each loaded with 9.5 mm stainless steel ball

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

Note: Consider cryogenic options for 4 mL & 15 mL tubes.

#2155
9.5 mm Stainless Steel Balls

#2240-PC
Polycarbonate Vial, 5 mL

## Conclusion

The Geno/Grinder® and MiniG® are proven and effective high-throughput tissue homogenizers. They are extremely versatile and have 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 optimize 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 Cole-Parmer representative or visit coleparmer.com.

## 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. Cole-Parmer wishes to thank Dave Burden for his assistance and permission to republish the document.*