

BULLET BLENDER® USER GUIDE: TABLE OF CONTENTS

GENERAL OPERATION		CONSIDERATIONS BY ANALYTE	
4	Considerations: RNA	27	
5	Considerations: DNA	28	
6	Considerations: Protein	29	
7	Bacterial & Viral Extraction	30	
8	Cell Dissociation	31	
9	Organelles	32	
10	Nanoparticles & Liposomes	33	
11	Reduce Particle Size	34	
SAMPLE PREPARATION & HANDLING		CONSIDERATIONS BY SAMPLE TYPES	
13	Protocol Library	36	
14	Organ Tissue	37	
15	Plant Matter	38	
16	Dried Grains, Nuts and Beans	39	
17	Pre-Crushing with the Stomper	40	
18	Small Organisms	41	
19	Insects	42	
20	Hair	43	
	Dehydrated Samples	44	
	Tissue Frozen in Liquid Nitrogen	45	
22	References & Publications	46	
23			
24			
25			
	5 6 7 8 9 10 11 13 14 15 16 17 18 19 20	4 Considerations: RNA 5 Considerations: DNA 6 Considerations: Protein 7 Bacterial & Viral Extraction 8 Cell Dissociation 9 Organelles 10 Nanoparticles & Liposomes 11 Reduce Particle Size CONSIDERATIONS BY SAMPLE TYPES 13 Protocol Library 14 Organ Tissue 15 Plant Matter 16 Dried Grains, Nuts and Beans 17 Pre-Crushing with the Stomper 18 Small Organisms 19 Insects 20 Hair Dehydrated Samples Tissue Frozen in Liquid Nitrogen References & Publications 22 23 24	



GENERAL OPERATION

PARTS OF THE BULLET BLENDER

> PART NAMES

- A. Cover
- B. Sample Tube
- C. Enclosure
- D. Gasket
- E. Sample Plate
- F. Operator Panel





GASKET FOR TUBE TYPE

NEXT ADVANCE
BULLET BLENDER

➤ NOTES

Bullet Blenders are fitted with a gasket that matches a specific kind of sample tube. Some machines have more than one gasket, which allows you to use different tube types.

Gaskets are labelled. Using the incorrect gasket may result in poor homogenization or prevent the cover from fully closing.





BULLET BLENDER® USER GUIDE PLACEMENT OF TUBES

> NOTES

There are no restrictions as to which holes to place your sample tubes in.

You do not need to balance your tubes as you would in a centrifuge.

However, you may get better results if you space them evenly.





BULLET BLENDER® USER GUIDE ADJUSTING THE SPEED & DURATION

> NOTES

Set the desired speed and time by adjusting the dials on the front of the instrument.

Homogenizing tougher tissue requires longer durations at full speed, while mixing or cell dissociation requires lower speeds. Suggested speeds and times for different tissues are available on the Next Advance Website.





AIR COOLING

> NOTES

Air CoolingTM, found on most Bullet Blender models, draws ambient air past tubes to reduce heating. Using the Bullet Blender in a cold room draws cold air past the sample tubes for better cooling.

When Gold or Gold Plus models are used without dry ice, operate them with the dry ice compartment open, so that they can draw in ambient air.





4 °C COOLING: OVERVIEW

> NOTES

Bullet Blender Gold and Gold Plus units cool samples by blowing air past dry ice.

The flow rate of the air is adjusted based on temperature sensor data and run cycle parameters, which ensures that samples do not freeze and will stay within a few degrees of 4°C.







4 °C COOLING: SELECTING DRY ICE

> NOTES

We recommend using 5/8" (1.5 cm) dry ice pellets.

Very fine pellets may block airflow and prevent proper cooling.

Larger chunks of dry ice have less surface area, so they are not as efficient.





4 °C COOLING: DRY ICE FILL AMOUNT

> NOTES

Overfilling the machine may block airflow.

Underfilling will result in inadequate cooling.

For proper fill volume, refer to fill line inside of interior bucket or to the image displayed.

PROPER FILL PROPORTION:









SAMPLE PREPARATION & HANDLING

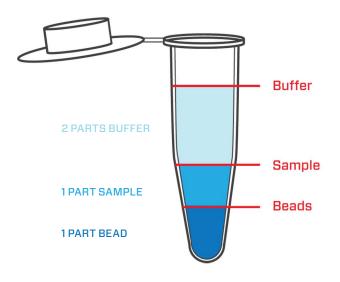
PROPER FILL PROPORTION

> NOTES

Proportions are volumetric.

Always keep the beadto-buffer ratio the same. Reduce the amount of sample if desired.

Different ratios may be required for certain sample types. See specific protocol for details.



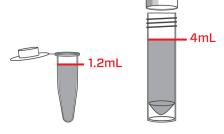


MAXIMUM TOTAL VOLUME

> NOTES

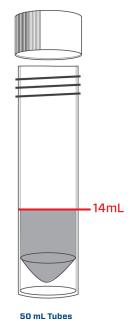
Overloading the tube will result in poor homogenization and can cause tube leakage.

Some sample types homogenize more efficiently with smaller tube loads.



5 mt Tubes

1.5-2 mL Tubes





BULLET BLENDER® USER GUIDE USE OF DETERGENT

> NOTES

Homogenizing samples in buffer containing detergent may result in excessive sample foaming.

We recommend adding detergent after homogenization. Foaming may also be reduced by lowering the homogenization speed, or by increasing the sample volume (if compatible with your experimental protocol).



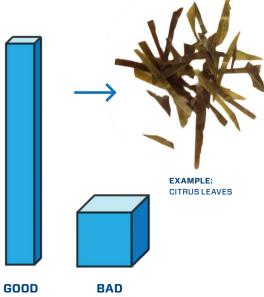


BULLET BLENDER® USER GUIDE CUTTING A SAMPLE PROPERLY

> NOTES

Long, thin samples will homogenize faster than cube-shaped or round samples.

To better homogenize tough samples, cut your sample into thin strips.





CELL CULTURE PREPARATION

> NOTES

Cells should be pelleted and then resuspended in the recommended volume of buffer before homogenization.

Total packed volume of cells should be 300 uL or less for microcentrifuge tube models, 1 mL or less for 5 mL tube models, and 3.5 mL or less for 50 mL tube models.



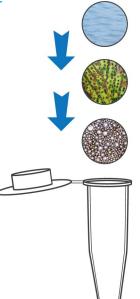


HOW TO LOAD A SAMPLE

NOTES

For most samples, add the beads to the tube, then the sample, and then the buffer. This prevents sample from being trapped in the narrow bottom of the tube, which can impair homogenization.

Special case: When using an 11 mm bead in the Bullet Blender 5 Storm, add the sample first and then place the bead on top of it.



3. BUFFER

2. SAMPLE

1. BEADS

NOTE:
DO NOT
WRAP
TUBE IN
PARAFILM.



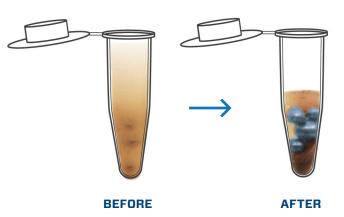
BULLET BLENDER® USER GUIDE RETRIEVING SAMPLES FROM TUBES

> NOTES

Soluble targets (protein, DNA, RNA, etc.) can be retrieved from the supernatant after centrifugation.

If you require whole homogenate, remove the beads with a Next Advance Magnetic Wand or carefully remove as much of the homogenate as possible from around the beads with a pipette.

CENTRIFUGATION





REMOVAL OF BEADS WITH MAGNETIC WAND

> NOTES

If using stainless steel beads, you can retrieve beads with a Next Advance Magnetic Wand, leaving only the homogenate in the tube.

NEXT ADVANCE MAGNETIC WAND







BEAD SELECTION

BEAD LYSIS KITS

> NOTES

Bead Lysis Kits are a convenient all-in-one solution: beads are pre-loaded into homogenization tubes. Just add sample and buffer.

Choose bead kits based on your sample type and size.





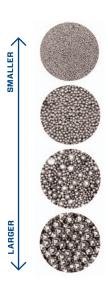
BEAD SELECTION: SIZE

> NOTES

Use beads that are approximately the same size as the sample you are trying to homogenize. Small beads are excellent for bacterial cultures or other cell suspensions, and larger beads are good for cut-up tissue.

Using beads that are too large for the tube (e.g, 4.8 mm beads in a microcentrifuge tube) can result in inefficient homogenization and tube failure.



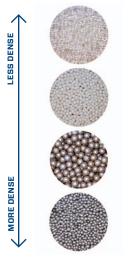


BEAD SELECTION: MATERIAL

NOTES

Use denser beads for tougher samples.

Lighter beads, such as glass, can be used for soft samples. Denser beads such as zirconium oxide or stainless steel provide more homogenizing power.



GLASS BEADS

ZIRCONIUM SILOCATE BEADS

ZIRCONIUM OXIDE BEADS

STAINLESS STEEL BEADS



BULLET BLENDER® USER GUIDE BEAD SELECTION: SPECIALTY BEADS

> NOTES

Use special bead types to homogenize difficult samples.

For resilient samples that contain a lot of connective tissue or fibers, consider using "UFO" beads. The sharper edges of these beads are excellent for cutting up tough samples.

To crush dry grains into powder, use large stainless steel beads (6 or 11 mm) in the Bullet Blender Storm 5.





STAINLESS STEEL UFO BEADS



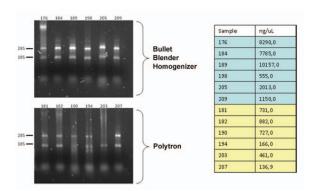
CONSIDERATIONS BY ANALYTE

CONSIDERATIONS: RNA

> NOTES

If it is important to keep your samples cold, consider Storm or Gold models with air cooling and dry ice cooling.

Use RNase-free beads and tubes to limit sample degradation. Consider RNAse-free Bead Lysis Kits to reduce handling.



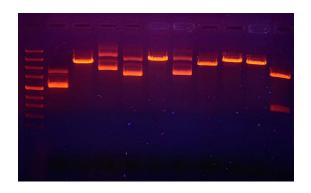


CONSIDERATIONS: DNA

> NOTES

The Bullet Blender does not cause excessive shearing of DNA during processing.

Full length chromatin can be extracted using the Bullet Blender at lower speeds.



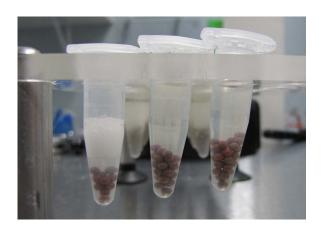


CONSIDERATIONS: PROTEIN

> NOTES

It is especially important to avoid foaming.

Storm or Gold model Bullet Blenders provide extra cooling, which is useful for samples containing heat-labile proteins.



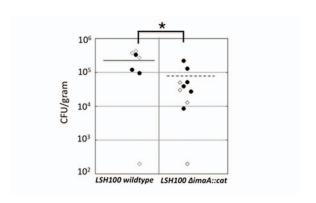


BULLET BLENDER® USER GUIDE BACTERIAL & VIRAL EXTRACTION

> NOTES

Bullet Blenders can be used to isolate bacteria and viruses from infected tissue and plant material.

Specific protocols vary by sample and analyte. Check our protocol webpage for examples.





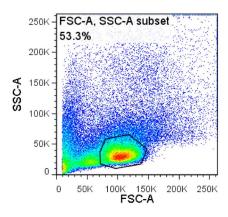
BULLET BLENDER® USER GUIDE CELL DISSOCIATION

> NOTES

Use large dense beads. Homogenize samples at low speeds.

There will be some loss of viability. Tougher samples have greater loss.

Use of a digestion buffer (e.g., containing collagenase) may aid in dissociation.



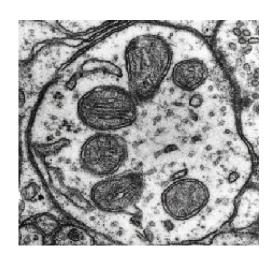


BULLET BLENDER® USER GUIDE ORGANELLES (E.G. NUCLEUS, MITOCHONDRIA, CHLOROPLASTS)

> NOTES

Some organelles, such as mitochondria, can be isolated from cells using a Bullet Blender.

To minimize damage to the organelles, use the gentlest conditions that still result in cell lysis.





NANO PARTICLES/LIPOSOMES

> NOTES

The Bullet Blender can be used to generate consistent nano scale particles or liposomes ranging from 50 - 100 nm.

Particle size of nanoparticles can be decreased by increasing homogenization time.



BULLET BLENDER® USER GUIDE REDUCE PARTICLE SIZE

> NOTES

Coarsely-ground material can be made into finer powders in a Bullet Blender, and it can also be used to break up clumps.

Even suspensions can be created by running the Bullet Blender at a high speed.

To finely grind material, use large stainless steel beads. To break up clumps, large stainless steel or zirconium oxide beads may be used.





CONSIDERATIONS BY SAMPLE TYPE

BULLET BLENDER® USER GUIDE PROTOCOL LIBRARY

> NOTES

Our experienced staff of molecular biologists have worked to provide you with a set of optimized protocols for the homogenization of various tissue, cell types, and organisms so you can spend less time troubleshooting and more time getting results.

The QR code links to the protocol page on our website.





BULLET BLENDER® USER GUIDE ORGAN TISSUE

> NOTES

Generally larger animals have "tougher" organs, so you may need to increase homogenization cycle times beyond protocol recomendations.

Connective tissue within your sample will take longer to homogenize.







BULLET BLENDER® USER GUIDE PLANT MATTER

> NOTES

For tough, fibrous plant tissue, consider using "UFO" beads, which are excellent for chopping fibers.

Some plant material homogenizes more efficiently if buffer volume is reduced.





DRIED GRAINS, NUTS AND BEANS

> NOTES

Some samples may need pre-crushing using the Next Advance Stomper. See specific protocol for details.

The Bullet Blender 5 Storm with an 11 mm stainless steel bead is the best choice for most samples of this type.







BULLET BLENDER® USER GUIDE PRE-CRUSHING WITH THE STOMPER

> NOTES

Hard samples like dried corn, soybeans and shells can be pre-crushed right in the sample tube.

Place the tube in the holder of the Next Advance stomper and pull the handle to crush.

Disposable shields can be used to prevent any cross-contamination.



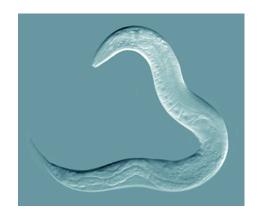


BULLET BLENDER® USER GUIDE SMALL ORGANISMS

> NOTES

Small soft organisms, like fruit flies and nematodes, can be homogenized in the same way tissue samples are.

- Place the samples into the tube dry, or spin them down at low speed and remove the supernatant if they were cultured in growth media.
- Add beads and buffer, flick the tubes lightly to resuspend if the samples are pelleted, and homogenize.





BULLET BLENDER® USER GUIDE INSECTS

NOTES

Heavy-bodied insects, such as fruit flies, can be homogenized in the same way as animal tissue.

Some insects, such as small ticks, can float on the buffer surface. To homogenize these samples, perform one run with just beads and sample, and then add buffer and re-homogenize to finish.





> NOTES

Do not densely pack hair into the sample tube. Overloading the tube will cushion the bead impacts and prevent good homogenization.

Hair is best homogenized dry. Buffer can be added after homogenization. Use 2.0 mm zirconium oxide beads and run at top speed.





BULLET BLENDER® USER GUIDE DEHYDRATED SAMPLES

NOTES

Dehydrated samples can be homogenized dry, to form a powder, or wet. For best results with wet homogenization, rehydrate the sample fully before processing.

Efficiency will be improved if the sample is cut into thin strips before homogenization.





BULLET BLENDER® USER GUIDE TISSUE FROZEN IN LIQUID NITROGEN

> NOTES

Immerse the frozen tissue in cold buffer and allow it to thaw, then treat it as you would any other sample.

If the tissue was dehydrated before it was frozen, you can pulverize the tissue by homogenizing with beads only (no buffer), then adding the cold buffer and running again to complete the homogenization.





BULLET BLENDER USER GUIDE REFERENCES & PUBLICATIONS

NOTES

The Bullet Blender has been used in a wide variety of applications for many years. Check our publication reference section to see if previous studies similar to yours are available. The Bullet Blender has been cited in more than 500 publications!



