BioMasher

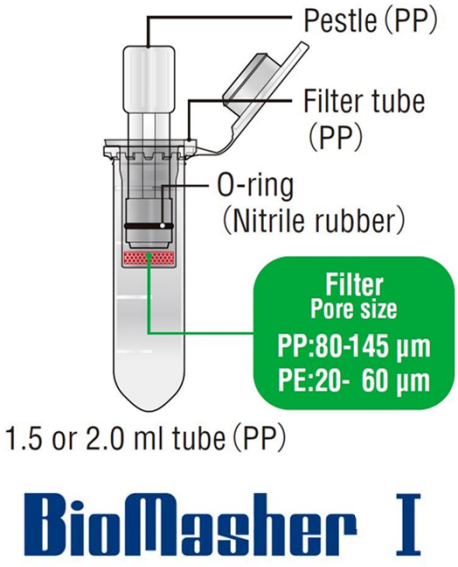
User Manual

BioMasher is a disposable homogenizer.

BioMasher is used to disrupt the tissue sample when extracting RNA, DNA, and proteins from the sample.
The BioMasher lineup includes the BioMasher I, II, III, V, and SP models.
BioMasher I has five variations: with or without an O-ring and by filter tube pore size.

BioMasher I

BioMasher I consists of a filter tube and a pestle. The sample is placed in the filter tube and the pestle is inserted from above. Centrifugal force is applied with a centrifuge allowing the pestle to crush the sample with this force. This allows the sample to pass through the filter of the filter tube, enabling the sample to be disrupted. The fibrous membrane component of the sample will be caught by the filter.
The pestle has a cross-shaped blade at the tip. By pushing and turning the pestle manually, the sample, which is hard to disrupt by centrifugation alone, can be ground.



BioMasher I

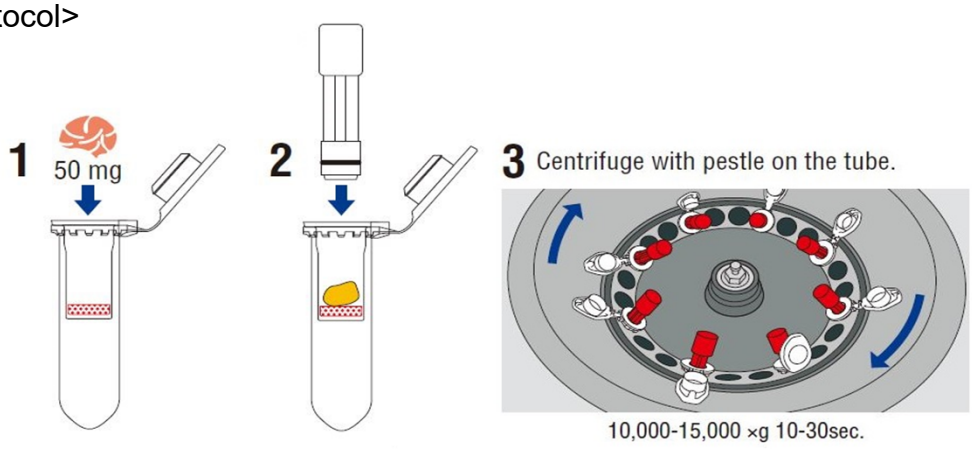
<Variation>
★ With an O-ring
BioMasher I with an O-ring is suitable for homogenizing soft tissue with centrifugal force alone. In particular, it is suitable for simultaneous processing of many samples and for infectious samples.

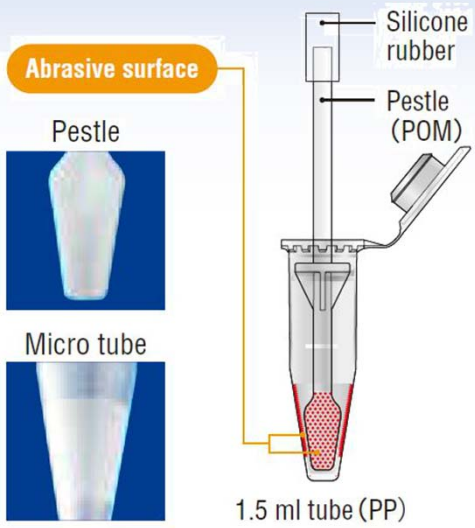
★ Without an O-ring
Hard tissue may not be homogenized sufficiently by centrifugal force alone. The model without an O-ring is suitable when grinding the sample on the filter area before centrifugation to enhance disruption efficiency.

∗ The material of the filter is available made of either polyethylene (PE) or polypropylene (PP).
The PE filter has smaller pore size than the PP filter resulting in finer disruption with the PE filter.

* DO NOT add the buffer in the filter tube before centrifuge. Otherwise the filter may be clogged, and the tube may be damaged. For only samples with lots of fibrous components with little water content, such as epidermis, mesentery, tail etc., add a small amount of buffer to extract and filtrate the target molecules.

<protocol>





BioMasher II

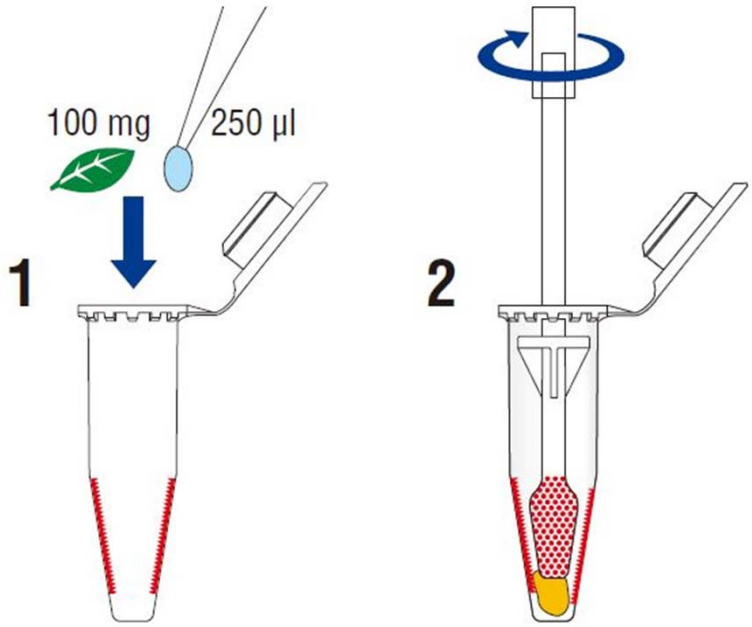
BioMasher II

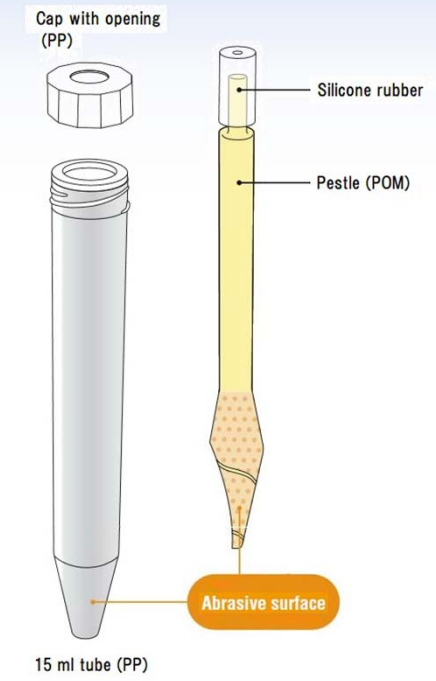
BioMasher II consists of a 1.5 mL microtube and a pestle. The sample and extraction buffer are placed in the tube, and ground to prepare the homogenate.
The microtube and pestle are molded so that they fit without any space between them. The texture of the inner wall of the tube and the outer periphery of the tip of the pestle enhances the efficiency of the disruption of the sample.
The pestle has a splash guard, which is designed to fit the tube without unnecessary spaces.
BioMasher II can be used to disrupt various samples, such as animal tissue/organ, plants (stems, roots, seeds), and insects.

★For samples that are difficult to disrupt, an exclusive electric stirrer (Nippi PowerMasher II) is available for easy disruption.

★DO NOT autoclave BioMasher II. In the case sterilization is required, please use a sterilized product.

<protocol>





BioMasher sp

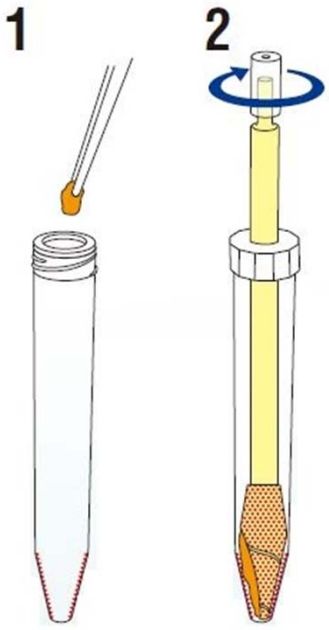
BioMasher SP

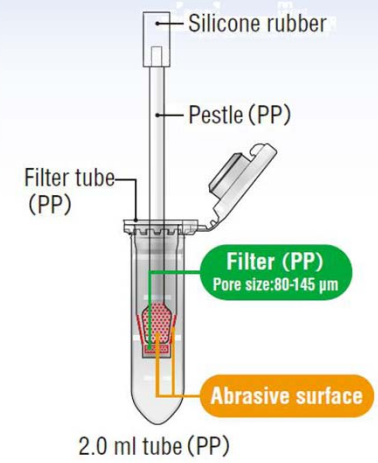
BioMasher SP is a larger volume version of BioMasher II. This model has enhanced disruption efficacy from the combination of the 15 mL tube with an abrasive inner wall and a stirring pestle with a textured tip.
The shovel-like shape cutout and the helical groove of the pestle effectively trap and homogenize the tissue.
BioMasher SP is suitable for disruption of animal tissue/organ, plants (stems, roots, seeds), and insects.

★For samples difficult to disrupt, an exclusive electric stirrer (Nippi PowerMasher II) is available for easy disruption.

★ DO NOT autoclave BioMasher II. In the case sterilization is required, please use a sterilized product.

<protocol>





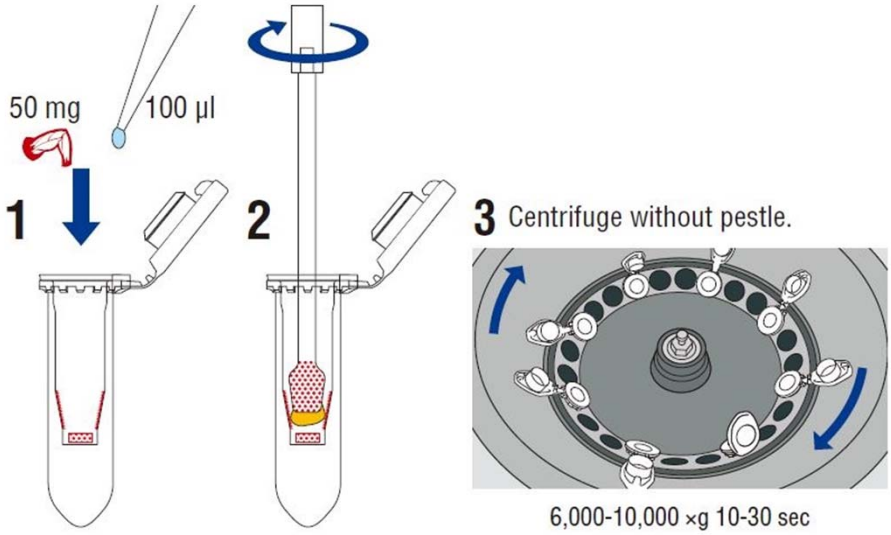
BioMasher III

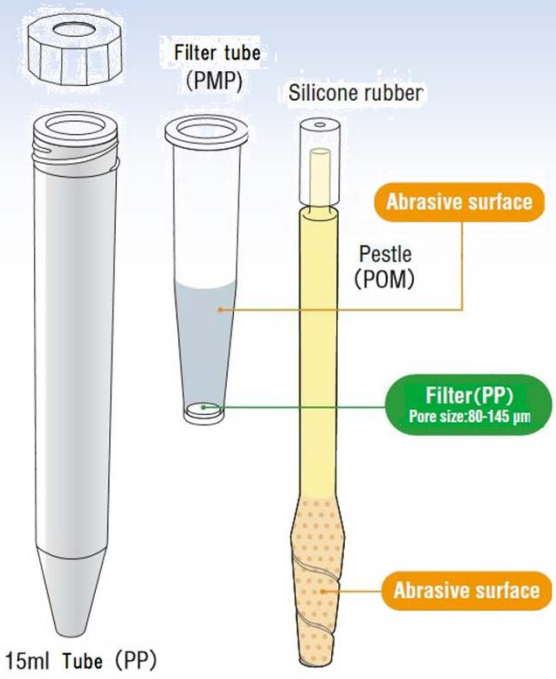
BioMasher III

BioMasher III has the features of both BioMasher I and II. This model consists of a filter tube with an abrasive surface inner wall and a pestle with a textured surface. After grinding the sample in the filter tube using the pestle, centrifuge the extraction liquid.
The extracted sample will be filtered and caught in the tube.
Since the fibrous membrane component get trapped by the filter, BioMasher III is recommended for homogenization of highly fibrous samples.

★For samples that are difficult to homogenize, an exclusive electric stirrer (Nippi PowerMasher II) can be used for ease.

<protocol>





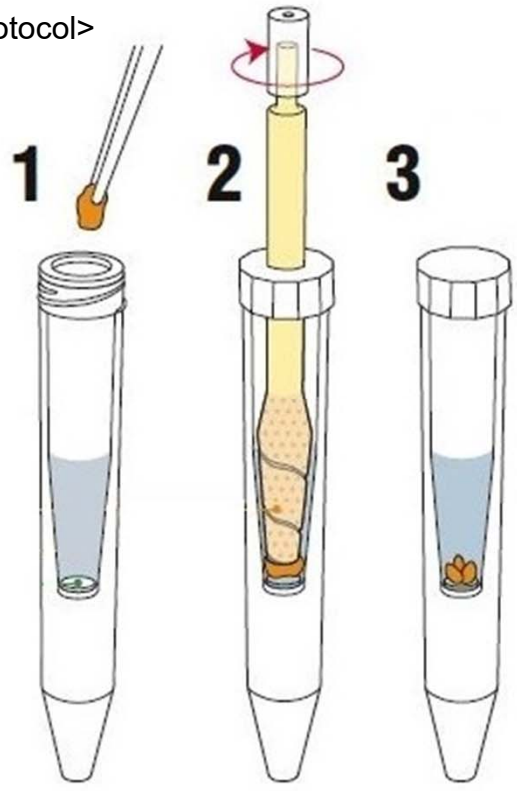
BioMasher V

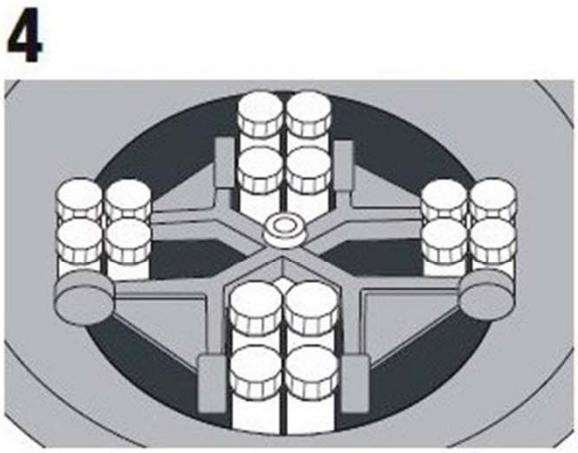
BioMasher V is a larger volume version of BioMasher III.
After homogenizing the sample with a combination of the tube with a textured inner wall and the stirring pestle with an abrasive surface, centrifuge and filtrate with the filter.

Recommended for homogenizing highly fibrous samples.

★For samples difficult to homogenize, an exclusive electric stirrer (PowerMasher II) is available for ease.

<protocol>





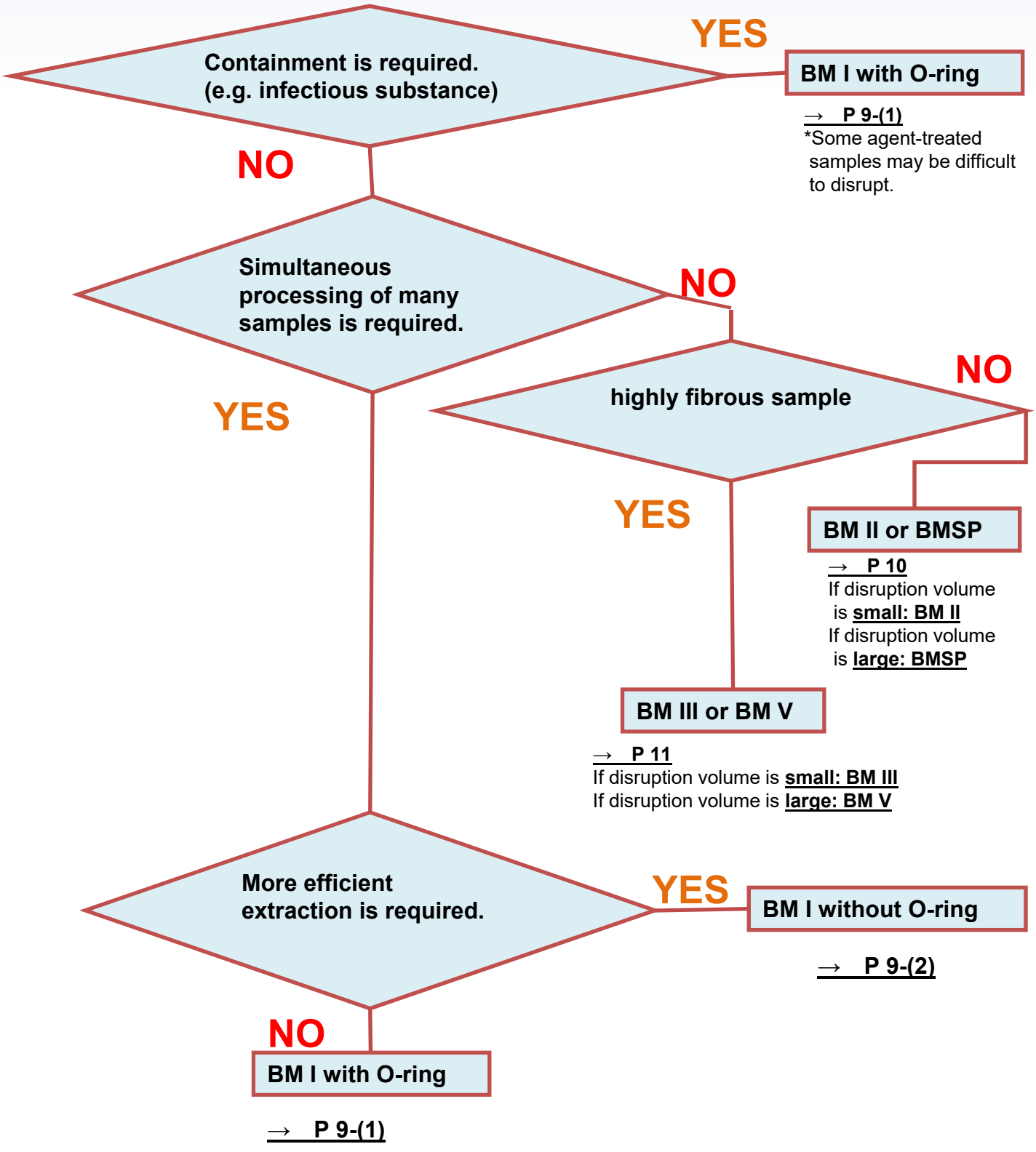
3,000-6,000 ×g 5min.

PowerMasher II helps the disruption of large amounts of samples and disruption that is difficult to complete manually. The device is attached to the stirring pestle of the BioMasher II, III, V, and SP to allow electrical stirring.
Geared high torque type with a rotation speed of 450 rpm.

*Two AA dry-cell batteries are required.

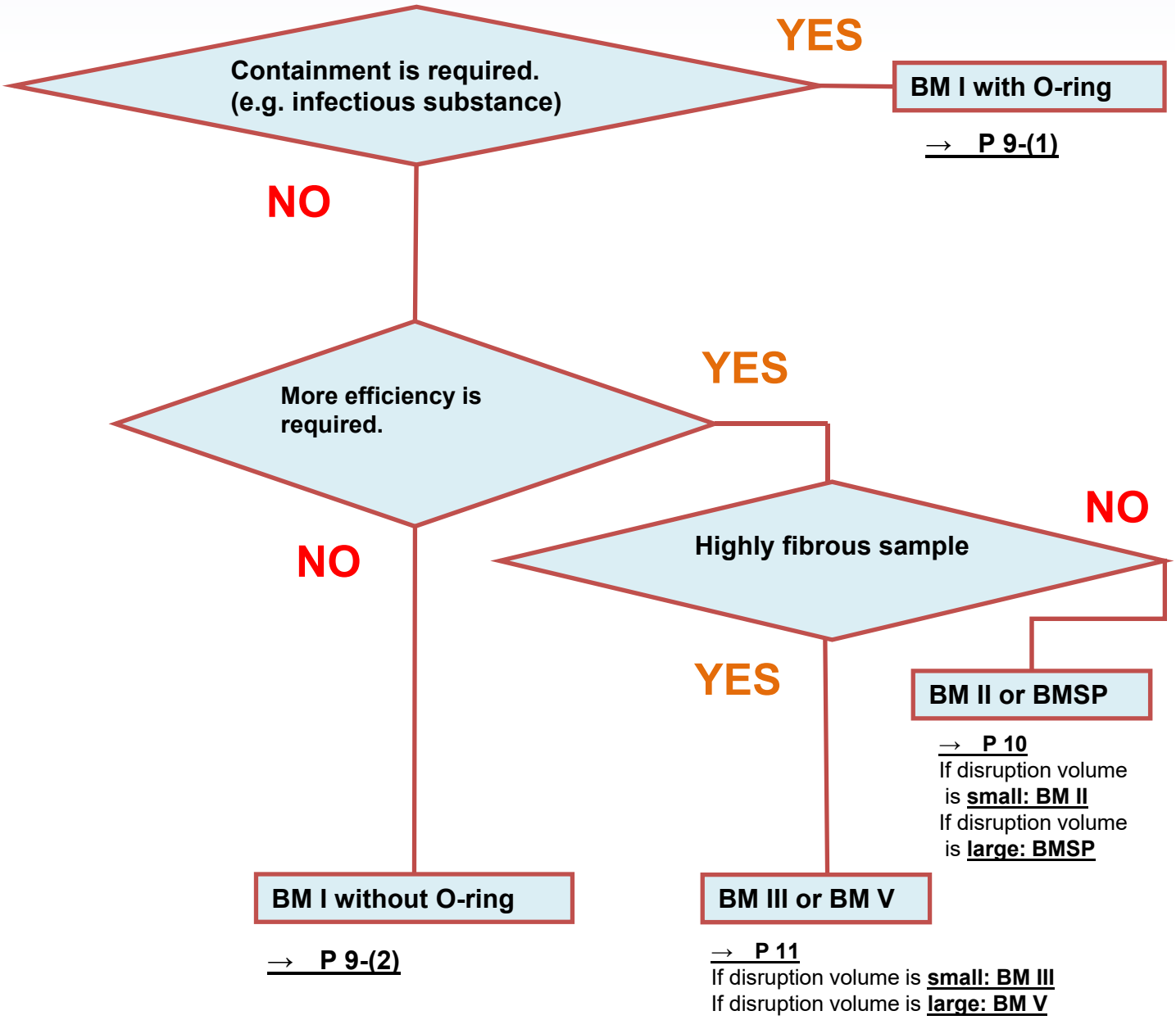
BioMasher Series Model Suitability by Tissue Type (1)

Soft Tissue — Brain, medulla oblongata, kidneys, liver, spleen, etc.



BioMasher Series Model Suitability by Tissue Type (2)

Hard Tissue — Epithelial tissue, digestive organs, heart, muscle, cartilage, etc.



BioMasher I Protocol

(1) For the liver, kidneys, cerebrum, cerebellum, and spleen (fresh sample)
→Use a pestle with an O-ring.

1. **Set a filter tube into the recovery tube and put 50–100 mg of the tissue to disrupt in the filter tube.**
2. **Insert the pestle with the O-ring into the filter tube and push it to the end.**
3. **Centrifuge at 15,000 x *g* for 30 seconds.**
4. **Discard the filter tube and pestle.**
5. **Add 1 mL of disruption buffer in the recovery tube and vortex.**

(2) For the small intestine, large intestine, lung, tail, muscle, seminal vesicle, gallbladder, salivary glands, preputial gland, heart, blood vessels, and agent-treated organs
→ Use a pestle without an O-ring.

1. **Set a filter tube into the recovery tube and put 50–100 mg of the tissue to disrupt in the filter tube.**
2. **Add 100 µL of disruption buffer in the filter tube and insert the pestle without an O-ring.**
3. **Disrupt the tissue by rotating the pestle while pushing it into the filter area of the filter tube.**
4. **Centrifuge at 15,000 x *g* for 30 seconds.**
5. **Discard the pestle and add 900 µL of disruption buffer to the filter tube.**
6. **Centrifuge at 15,000 g for 30 seconds.**
7. **Discard the filter tube.**
8. **Close the cap of the recovery tube and vortex.**

References which BioMasher I is used.

1) Yamamoto, T. et al. (2012) Improved RNA extraction method using the BioMasher and BioMasher power-plus. Journal of Veterinary Medical Science, 74: 1561-7
2) Yamamoto, T. et al. (2013) Sensitivity and specificity of a commercial BSE kit for the detection of ovine scrapie. Animal Science Journal, 84: 508-12
3) Aboelenain, M. et al. (2015) Status of autophagy, lysosome activity and apoptosis during corpus luteum regression in cattle. Journal of Reproduction and Development, 61: 229-36
4) Lee, B.S. et al. (2017) Hypothermia decreased the expression of heat shock proteins in neonatal rat model of hypoxic ischemic encephalopathy. Cell Stress and Chaperones, 22: 409–15
5) Ikeda, R. et al. (2015) Knockdown of aquaporin-8 induces mitochondrial dysfunction in 3T3-L1 cells. Biochemistry and Biophysics Reports, 4: 187-95
6) Uchida, T. et al. (2017) Abnormal γ-aminobutyric acid neurotransmission in a Kcnq2 model of early onset epilepsy. Epilesia, 58: 1430-39
7) Nakayama, T. et al. (2017) Detection of fungi from an indoor environment using loop-mediated isothermal amplification (LAMP) Method. Biocontrol Science, 22: 97-104
8) Roberts, B.R. et al. (2017) Biochemically-defined pools of amyloid-β in sporadic Alzheimer's disease: correlation with amyloid PET. Brain, 140: 1486–98

BioMasher II Protocol

Samples to disrupt: Animal tissues/organs, plants (stems, roots, seeds), insects, etc.

1. **Put 50–100 mg of tissue to disrupt in the tube included in BioMasher II.**
2. **Add 250 µL of disruption buffer.**
3. **Insert the pestle and disrupt the tissue while pressing the pestle to the side of the tube.**
4. **Discard the pestle and add 750 µL of disruption buffer.**
5. **Vortex.**

BioMasher SP Protocol

Samples to disrupt: Animal tissues/organs, plants (stems, roots, seeds), insects, etc.

1. **Put the tissue to disrupt in the tube included in BioMasher SP.**
2. **Add disruption buffer.**
3. **Insert the pestle and disrupt the tissue while pressing the pestle to the side of the tube.**
4. **Discard the pestle and add disruption buffer.**
5. **Vortex.**

References which BioMasher II or SP is used.

1) Jinnai, M. et al. (2017) Production of a novel monoclonal antibody applicable for an immunochromatographic assay for Kudoa septempunctata spores contaminating the raw olive flounder (Paralichthys olivaceus). International Journal of Food Microbiology, 259: 59-67
2) Jasper Elvin James et al. (2016) In vitro antifungal susceptibility of neoscytalidium dimidiatum cinical isolates from malaysia. Mycopathologia, 182: 305–13
3) Gilbert, J. R., et al. (2017) Resequencing of the Col1A1 gene of Oryctolagus cuniculus identifies splicing errors and single nucleotide polymorphisms. Genes & Genomics, 39: 549–55
4) Kobayashi, D. et al. (2017) Detection of a novel putative phlebovirus and first isolation of Dugbe virus from ticks in Accra, Ghana. Ticks and Tick-borne Diseases, 8: 640-45
5) Kwon, D.H. et al. (2017) Genetic diversity and structure in apple-infesting pests of Carposina sasakii, Grapholita dimorpha and Grapholita molesta in Korea. Journal of Asia-Pacific Entomology, 20: 13-16
6) Isobe, K. et al. (2017) Platelet-rich fibrin prepared from stored whole-blood samples. International Journal of Implant Dentistry, 2017: 3-6
7) Ishizawa, H. et al. (2017) Differential oxidative and antioxidative response of duckweed Lemna minor toward plant growth promoting/inhibiting bacteria. Plant Physiology and Biochemistry, 118: 667-73
8) Matsubara, T. et al. (2017) Regulation of osteoclast differentiation and actin ring formation by the cytolinker protein plectin. Biochemical and Biophysical Research Communications, 489: 472–76

BioMasher III Protocol

Samples to disrupt: Among animal tissues/organs, plants (stems, roots, seeds), insects, etc., samples with much fiber

1. **1.Put the sample in BioMasher III and disrupt it with 100 µL of disruption buffer.**
2. **2.Wash out the tissue section attached to the pestle with 200 µL of disruption buffer in the filter tube.**
3. **Discard the pestle and centrifuge at 10,000 x g for 30 seconds at room temperature.**
4. **Add 700 µL of disruption buffer.**
5. **Vortex.**

BioMasher V Protocol

Samples to disrupt: Among animal tissues/organs, plants (stems, roots, seeds), insects etc., samples with much fiber

1. **Put the sample in BioMasher V and disrupt it with disruption buffer.**
2. **Wash out the tissue section attached to the pestle with disruption buffer in the filter tube.**
3. **Discard the pestle and centrifuge at 6,000 *g* for 30 seconds at room temperature.**
4. **Add disruption buffer.**
5. **Vortex.**

References which BioMasher III or V is used.

1) Alqaydi, M. et al. (2016) Quantitative and qualitative study of STR DNA from ethanol and formalin fixed tissues. Forensic Science International, 262: 18-29
2) Yoshizawa, T. et al. (2016) Effects of adrenomedullin on doxorubicin-induced cardiac damage in mice. Biological and Pharmaceutical Bulletin, 39: 737-46
3) Kimoto, M. et al. (2016) DNA aptamer generation by genetic alphabet expansion SELEX (ExSELEX) using an unnatural base pair system. Nucleic Acid Aptamers, Part of the Methods in Molecular Biology book series, MIMB, 1380: 47-60

FAQ

Q1. In terms of sample volume, which model of the BioMasher is appropriate?

→ Our recommendation is as below.

Sample size
BM I: 50 mg
BM II: 100 mg
BM III: 50 mg
BM V: 500 mg
BM SP: 1,000 mg

Q2. What is the centrifugation resistance of the BioMasher?

→ Although it depends on sample, Please try the following conditions as a standard.
BM I: 10,000-15,000 *g*, 10-30 seconds
BM II: 10,000-15,000 *g*, 10-30 seconds
BM III: 6,000-10,000 *g*, 10-30 seconds
BM V: 3,000-6,000 *g*, 5 minutes
BM SP: 3,000-6,000 *g*, 5 minutes

Q3. What is the lowest temperature BioMasher can bear?

→ The material of the tube is PP, and its freezing resistance is approximately -60°C to -40°C.
Cooling of the sample with liquid nitrogen together with the tube may cause damage.

Q4. Contact for other inquiries

→ Please access the following URL, which has the link to the inquiry form.

https://www.nippi-inc.co.jp/tabid/143/Default.aspx

2017/8/25 version

BioMasher I-III
Technical Document

1. Advantages of BioMasher II
2. RNA extraction experiment using BioMasher III
3. Comparison of RNA extraction efficiency among BioMasher I, II, and III
4. Protocol for RNA extraction from mouse tissue using BioMasher I
5. Protocol for RNA extraction from mouse tissue using BioMasher II and III

Nippi, Incorporated
Biological and Chemical Products Division
1-1-1 Senju Midori-cho, Adachi, Tokyo 120-8601, Japan
Tel: +81-3-3888-5184, Fax: +81-3-3888-5136

Disposable homogenizer

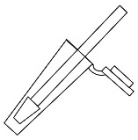
Advantages of BioMasher II

Harvest mouse liver.

Prepare the same amount of liver tissue (80 mg for each).

insufficient homogenization
Sample slips in the tube.
Sample scatters to the wall of the tube.
Time and labor are required for sufficient homogenization
(Sample is degrading.)

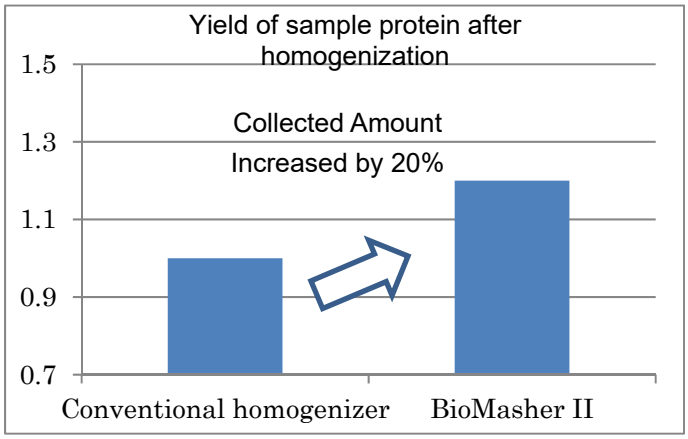
Add buffer and homogenize.



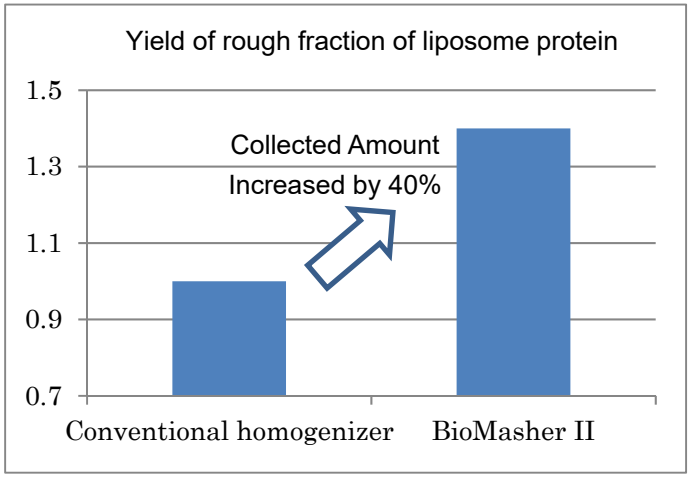
Conventional homogenizer

BioMasher II

Centrifuge and collect sup.
(Remove fat and ppt.)



Sucrose Density Gradient Ultracentrifugation
(Collects rough fraction of liposome protein by sucrose density gradient ultracentrifugation)



Pestle with abrasive surface

Microtube with abrasive inner surface.

Abrasive surfaces of the pestle and the microtube allow for sufficient homogenization in a rapid and simple manner

Pestle includes splashing prevention mechanism

Sufficient homogenization is completed rapidly and easily.
(Suppresses degradation of sample.)

RNA extraction experiment using BioMasher III

Nippi, Incorporated
Biological and Chemical Products Division

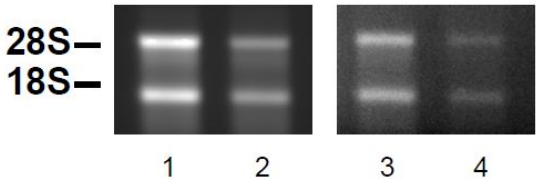
By BioMasher III, total RNA was extracted from liver, kidney, heart, and skeletal muscle of a mouse tissues are stored in RNAlater (Ambion).
The extraction protocol is as follows.

1. Put the tissue into the BioMasher III tube with 100 mkL of TRIzol(Thermo Fisher Scientific) and homogenize.
2. Wash the pestle with 200 µL of TRIzol.
3. Discard the pestle and incubate 3 minutes at room temperature.
4. After centrifugation at 12,000 rpm for 30 seconds, discard the filter tube, add 700 µL of TRIzol, and mix.
5. Add 200 µL of chloroform and incubate 3 minutes at room temperature.
6. After centrifugation at 12,000 rpm for 15 minutes at 8℃, transfer the supernatant to the new tube.
7. Add 500 µL of isopropyl alcohol and incubate 10 minutes at room temperature.
8. After centrifugation at 12,000 rpm for 10 minutes at 8℃, discard the supernatant.
9. Add 1 mL of 75% EtOH.
10. After centrifugation at 7,500 rpm for 5 minutes at 8℃, discard the supernatant and air dry for 5 minutes at room temperature.
11. Add 50 µL of DEPC treated water and dissolve for 10 minutes at 60℃.
12. Measure the RNA content by the absorbance at 260 nm.

Tissue	Tissue weight (mg)	mRNA (µg/mL; 50 µL)	Extraction Ratio (µg/mg)	260 nm/280 nm
1. Liver	36	948.84	1.32	1.91
2. Kidney	10	931.84	4.66	1.8
3. Heart	35	352.86	0.50	1.81
4. Skeletal Muscle	50	236.52	0.24	1.66

Extraction was more effective with BioMasher III compared with conventional SSI pestle.
With SSI pestle, extraction ratio of liver, Kidney, Heart and Muscle are 0.92, 0.15,0.12, and 0.43, respectively.

A agarose gel electrophoresis was performed, and the 28S and 18S bands were evaluated (RNA 1 µg/lane).



Both the 18S and 28S bands were observed in all tissues.

Comparison of RNA extraction efficiency among BioMasher I, II, and III

Nippi, Incorporated
Biological and Chemical Products Division

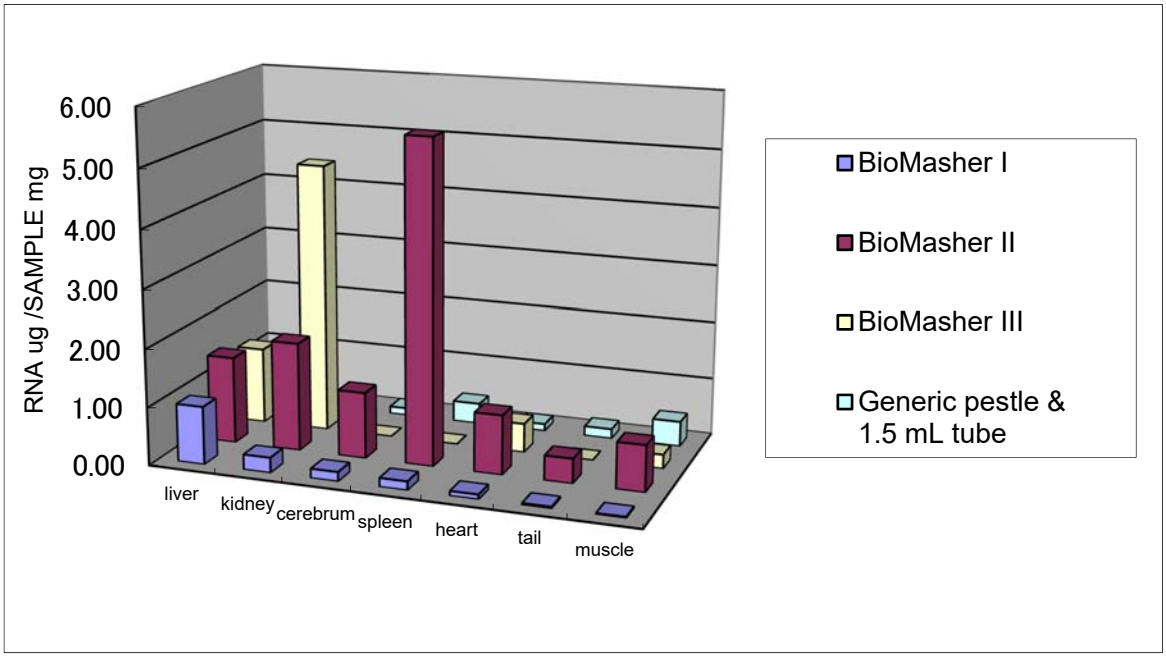
[Method]

RNA was extracted from various organs of a mouse (Crlj:CD1 [ICR]: Adult mouse of 8 weeks or older) using BioMasher I–III according to the attached protocol. As a control, RNA was extracted by using a generic pestle and a 1.5 mL tube.

[Results]

The results are summarized below.

Tissue	RNA Extraction Ratio (RNA (μg)/Sample (mg))			
	BioMasher I	BioMasher II	BioMasher III	Generic Pestle & 1.5 mL tube
Liver	1.00	1.50	1.32	0.92
Kidney	0.26	1.87	4.66	0.15
Cerebrum	0.16	1.14	-	0.12
Spleen	0.15	5.53	-	0.36
Heart	0.08	1.02	0.50	0.12
Tail	0.03	0.43	-	0.16
Muscle	0.02	0.80	0.24	0.43



*Choose appropriate model of BioMasher for the maximum extraction ratio of RNA depending on tissue type.

Protocol for RNA Extraction from Mouse Tissue Using BioMasher I

Nippi, Incorporated
Biological and Chemical Products Division

Use a pestle with or without an O-ring of BioMasher I depending on the hardness of the tissue.

For liver, kidney, cerebrum, cerebellum, and spleen (relatively soft tissues)

→ Use a pestle with an O-ring.

1	Set a filter tube into the recovery tube and put 50–100 mg of the tissue in the filter tube.
2	Insert the pestle with the O-ring into the filter tube and push it to the end.
3	Centrifuge at 15,000 x g for 30 seconds.
4	Discard the filter tube and pestle.
5	Add 1 mL of TRIzol in the recovery tube and vortex.

Follow TRIzol protocol.

6	Incubate for 5 minutes at 15ºC to 30℃.
7	Add 0.2 mL of chloroform, cap the tube, and mix by inverting by hand for 15 seconds.
8	Incubate for 2–3 minutes at 15ºC to 30℃.
9	Centrifuge at 12,000 x g for 15 minutes at 2ºC to 8℃.
10	Transfer the top RNA layer to the new tube.
11	Add 0.5 mL of isopropyl alcohol and incubate for 10 minutes at 15ºC to 30℃.
12	Centrifuge at 12,000 x g for 10 minutes at 2ºC to 8℃.
13	Discard the supernatant and add 1 mL of 75% ethanol.
14	Vortex and centrifuge at 8,000 x g for 5 minutes at 2ºC to 8℃.
15	Discard the supernatant and air dry for 5–10 minutes.
16	Add 50 µL of DEPC treated water or TE buffer and incubate for 10 minutes at 55ºC to 60℃.

For the small intestine, large intestine, lung, tail, muscle, seminal vesicle, gallbladder, salivary glands, preputial gland, heart, and blood vessels (relatively hard tissues)

→ Use a pestle without the O-ring.

1	Set a filter tube into the recovery tube and put 50–100 mg of the tissue in the filter tube.
2	Add 500 µL of TRIzol in the filter tube and insert the pestle without the O-ring.
3	Disrupt the tissue by rotating the pestle while pushing it into the filter tube.
4	Centrifuge at 15,000 x g for 30 seconds.
5	Discard the pestle and add 500 µL of TRIzol to the filter tube.
6	Incubate for 5 minutes at 15ºC to 30℃.
7	Centrifuge at 15,000 x g for 30 seconds.
8	Discard the filter tube
9	Close the cap of the recovery tube and vortex.

Follow TRIzol protocol. (Nos. 6 to 16 above)

Protocol for RNA extraction from mouse tissue using BioMasher II/III

Nippi, Incorporated

Biological and Chemical Products Division

BioMasher II

1	Put 50–100 mg of tissue in the tube included in BioMasher II.
2	Add 500 µL of TRIzol.
3	Insert the pestle into the tube and disrupt the tissue while pressing the pestle to the side of the tube.
4	Discard the pestle and add 500 µL of TRIzol.
5	Vortex.

Follow the TRIzol protocol.

6	Incubate for 5 minutes at 15ºC to 30℃.
7	Add 0.2 mL of chloroform, cap the tube, and mix by inverting by hand for 15 seconds.
8	Incubate for 2–3 minutes at 15ºC to 30℃.
9	Centrifuge at 12,000 x g for 15 minutes at 2ºC to 8℃.
10	Transfer the top RNA layer to the new tube.
11	Add 0.5 mL of isopropyl alcohol and incubate for 10 minutes at 15ºC to 30℃.
12	Centrifuge at 12,000 x g for 10 minutes at 2ºC to 8℃.
13	Discard the supernatant and add 1 mL of 75% ethanol.
14	Vortex and centrifuge at 8,000 x g for 5 minutes at 2ºC to 8℃.
15	Discard the supernatant and air dry for 5–10 minutes.
16	Add 50 µL of DEPC-processed water or TE buffer and incubate for 10 minutes at 55ºC to 60℃.

BioMasher III

1	Put the sample in BioMasher III and disrupt the sample with 100 µL of TRIzol.
2	Wash out the tissue section attached to the pestle with 200 µL of TRIzol in the filter tube.
3	Discard the pestle and allow to stand for 3 minutes at room temperature.
4	Add 700 µL of TRIzol after centrifugation at 12,000 rpm for 30 seconds at room temperature.
5	Vortex.

Thereafter follow the TRIzol protocol.

6	Incubate for 5 minutes at 15ºC to 30℃.
7	Add 0.2 mL of chloroform, cap the tube, and mix by inverting by hand for 15 seconds.
8	Incubate for 2–3 minutes at 15ºC to 30℃.
9	Centrifuge at 12,000 x g for 15 minutes at 2ºC to 8℃.
10	Transfer the top RNA layer to the new tube.
11	Add 0.5 mL of isopropyl alcohol and incubate for 10 minutes at 15ºC to 30℃.
12	Centrifuge at 12,000 x g for 10 minutes at 2ºC to 8℃.
13	Discard the supernatant and add 1 mL of 75% ethanol.
14	Vortex and centrifuge at 8,000 x g for 5 minutes at 2ºC to 8℃.
15	Discard the supernatant and air dry for 5–10 minutes.
16	Add 50 µL of DEPC-processed water or TE buffer and incubate for 10 minutes at 55ºC to 60℃.