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BioMasher

BioMasher I-III
Technical Document

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Disposable homogenizer

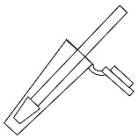
Advantages of BioMasher II

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.)

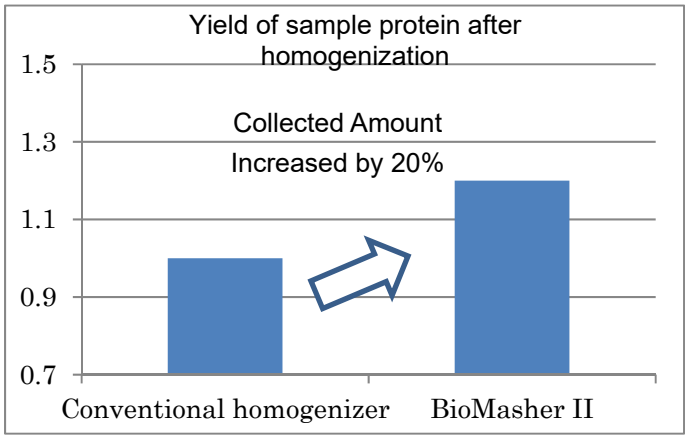
Add buffer and homogenize.



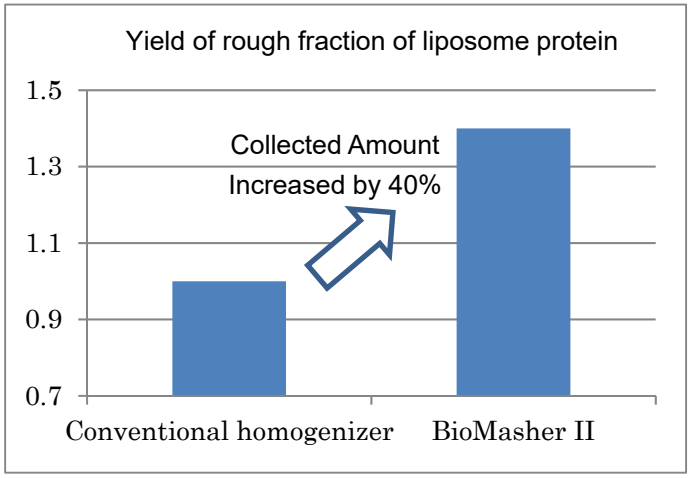
Conventional homogenizer

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

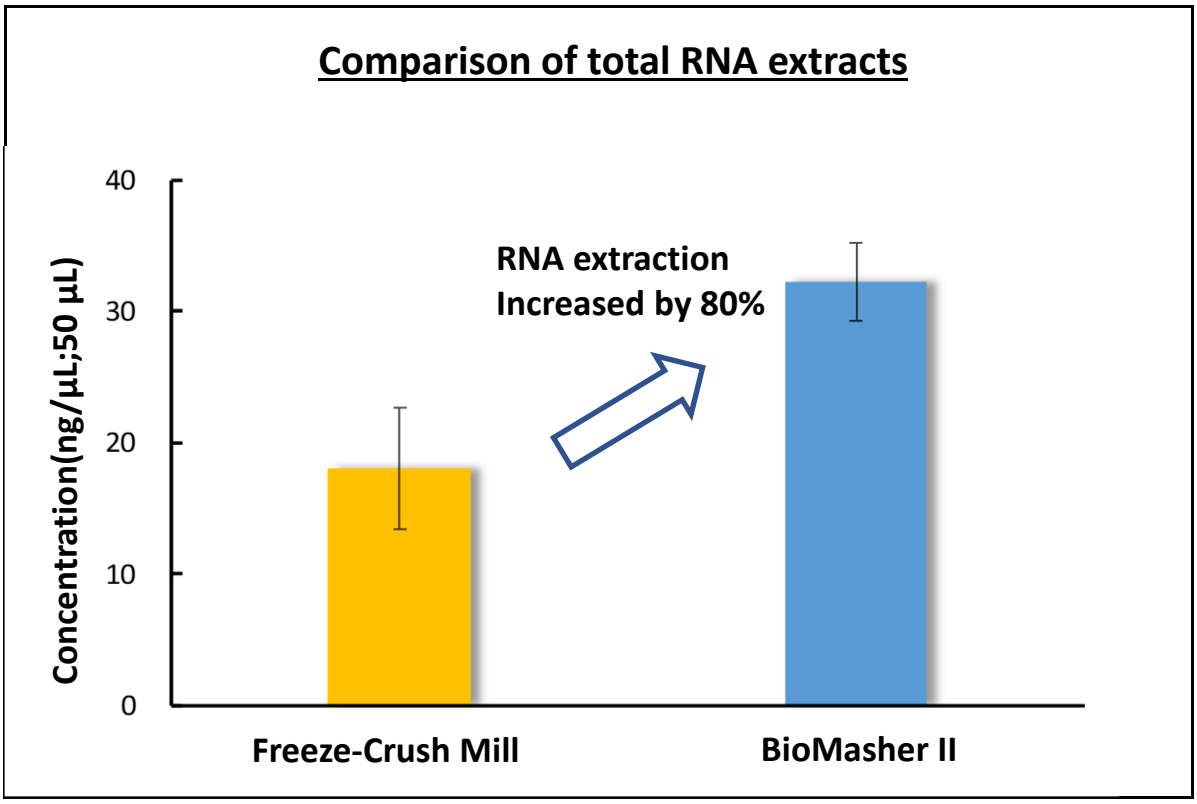
Sufficient homogenization is completed rapidly and easily.
(Suppresses degradation of sample.)

Extraction of Total RNA from beetle-derived antennae using BioMasherII

Nippi, Incorporated
Biological and Chemical Products Division

[Method]
BioMasher II dramatically improves the efficiency of total RNA extraction.
Using BioMasher II, RNA was extracted from the antennae of a beetle after emergence via the Relia Prep Nucleic Acid Purification System (Promega). The control experiment was performed using a freeze-crush mill and 2-ml tube.Total RNA was eluted with 50 μL of nuclease-free water from the purifying column and the absorbance was measured at 260 nm.

[Results]
The results are summarized below.



RNA extraction experiment using BioMasher III

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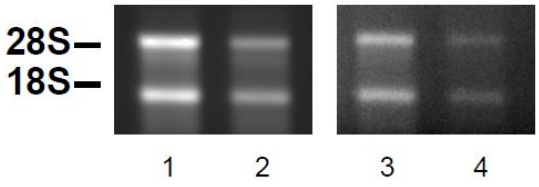
By BioMasher III, total RNA was extracted from liver, kidney, heart, and skeletal muscle of a mouse tissues are stored in RNAlater (Ambion).
The extraction protocol is as follows.

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

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Biological and Chemical Products Division

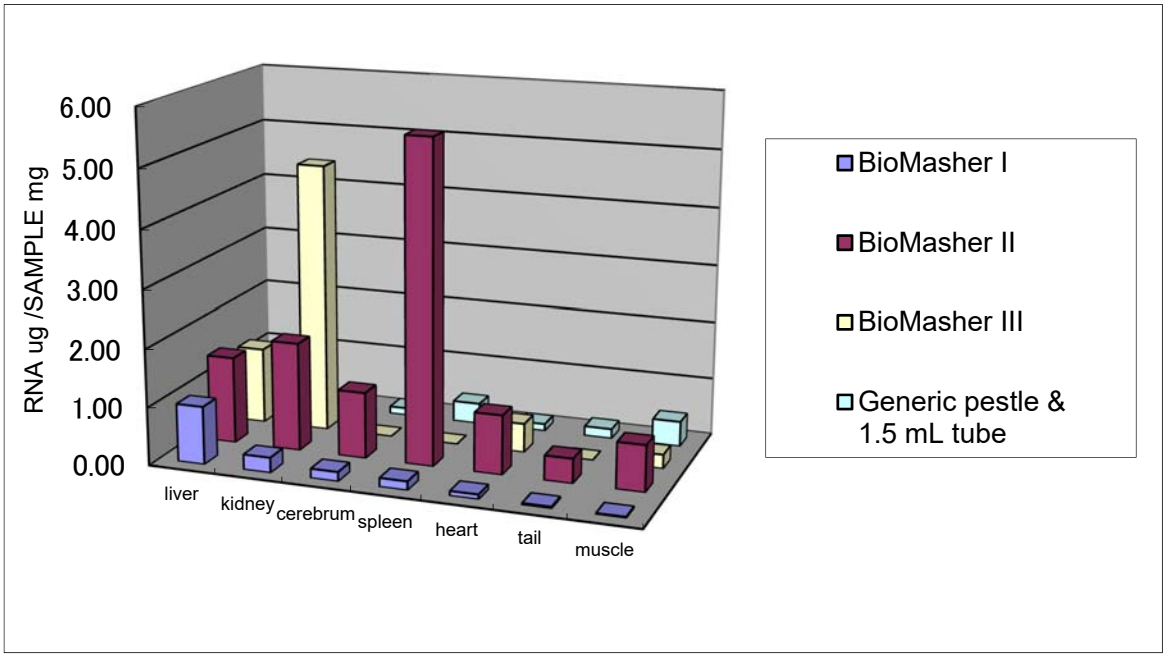
[Method]

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

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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

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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℃.