A&A BIOTECHNOLOGY
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Manual

Bead-Beat Total RNA Mini

Versatile kit for total RNA purification from various, lysis-resistant sources. Mechanical lysis procedure.

catalog #	size
031-25BB	25 isolations
031-100BB	100 isolations

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Specification

form	minicolumn
binding capacity	100 µg of RNA
sample size	• up to 3 ml of bacterial or yeast culture • up to 1 ml of yeast culture • up to 50 mg of plant or animal tissue
elution volume	from 100 µl
elution solution	ultrapure water

Contents

component	25 isolations	100 isolations	storage
Minicolumns	25 pcs	100 pcs	15–25 °C
2 ml tubes	25 pcs	100 pcs	15–25 °C
Bead-beat tubes (zirconia/silica beads)	25 pcs	100 pcs	15–25 °C
A1 wash solution	50 ml	200 ml	15–25 °C
Fenozol	25 ml	100 ml	2–8 °C
Isopropanol	15 ml	50 ml	15–25 °C
Ultrapure water	8 ml	15 ml	-20–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Beadbeater (BioSpec or MP Biomedicals)
- Chloroform
- Microcentrifuge
- Heatblock or incubator set to 50 °C

Optional

- DNAse (cat. # 1009-10, 1009-100)
- Clean-Up RNA Concentrator (cat. # 039-25C, 039-100C)

Important notes

When working with RNA, use RNAse-free consumables. Work sterile, use disposable gloves and change them whenever good laboratory practice requires it.

Material preparation

Fenozol deactivates endogenous RNAses. Sample suspended in fenozol can be stored:

- at -20 °C, -80 °C up to one year
- from +2 °C to +8 °C up to one week
- in room temperature up to 24 hours

Fenozol contains phenol. Avoid contact with skin. Wear suitable protective gloves.

Bacteria culture

1. Centrifuge **1-3 ml** of overnight bacterial culture. Discard the supernatant.
2. Add **800 µl** of **fenozol**, suspend the pellet and transfer the mixture to the bead-beat tube.
3. Follow point 1. of the protocol.

Yeast culture

1. Centrifuge **1 ml** of yeast culture. Discard the supernatant.
2. Add **800 µl** of **fenozol**, suspend the pellet and transfer the mixture to the bead-beat tube.
3. Follow point 1. of the protocol.

Animal / plant tissue

Note:
Not all tissue types are suitable for efficient bead beating disintegration. This is the matter of a preliminary experiment. The bead beating is suggested for soft tissues:
animal: e.g. brain, soft organs tissues, soft muscle, meat, fish muscles, etc.
plant: e.g. green soft stalk, soft leaf or soft root cuts.

1. Transfer **20-50 mg** pieces of soft tissue sample to the bead-beat tube.
2. Add **800 µl** of **fenozol**.
3. Follow point 1. of the protocol.

Isolation protocol

1. Lyse the samples by bead beating in a suitable Beatbeater. The bead beating time should be enough to disintegrate the material, but not to cause excessive warm up of the samples,
   e.g. 10-20 s interrupted by 1 min break to enable cooling down.
2. Incubate sample for **5 min** at **50 °C**.
3. Add **200 µl** of **chloroform** (not included) and gently mix by inverting the tube a few times.
4. Keep the sample for **3 min** at **room temp**.
   Centrifuge the sample for **10 min** at **10 000-12 000 RPM**.
5. Transfer the supernatant (**~ 450 µl**) to **a new** 1.5 ml tube (not included).

   Ensure not to withdraw the interphase that mainly contains the unwanted DNA fraction.

   Add **250 µl** of **isopropanol**.

   If there is a particular interest in the low molecular weight fraction of RNA, increase total isopropanol volume to 450 µl.
6. Thoroughly mix and apply onto the minicolumn.
7. Centrifuge for **1 min** at **10 000-12 000 RPM**.
8. Transfer the minicolumn to **a new** 2 ml tube (included).
9. Add **700 µl** of **A1** wash solution.
10. Centrifuge for **1 min** at **10 000-12 000 RPM**.
11. Remove the minicolumn from the tube and discard the filtrate.
    Place the minicolumn into the same tube. Add **700 µl** of **A1** wash solution.
12. Centrifuge for **1 min** at **10 000-12 000 RPM**.
13. Remove the minicolumn from the tube and discard the filtrate.
    Place the minicolumn into the same tube. Add **200 µl** of **A1** wash solution.
14. Centrifuge for **2 min** at **10 000-12 000 RPM**.

15. Transfer the dry minicolumn to **a new** 1.5 ml elution tube (not included).
Add **100 µl** of **ultrapure water** directly onto the minicolumn resin.

16. Keep for **3 min** at **room temp.**
Centrifuge for **1 min** at **10 000-12 000 RPM**.

17. Remove the minicolumn and store the tube with purified RNA at **-20 °C**, **-80 °C** until later use.

Additional clean-up / concentration of isolated RNA sample (optional)

Bead-Beat Total RNA Mini kit effectively isolates and purifies RNA for most downstream applications.

In case of the highest possible RNA sample purity being required, as for example supreme DNA removal, we recommend to additionally process RNA sample, as follows:

Use of the DNAse (cat. # 1009-10, 1009-100)

1. **To 100 µl** of **RNA eluate** add:

   1 µl of **DNAse** (10 U/µl)
   10 µl of **10x reaction buffer** (included with DNAse)

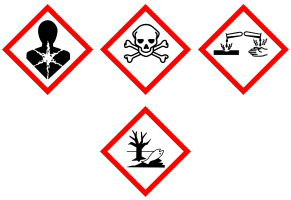
2. Incubate for **15 min** at **37 °C**.

3. Incubate for **10 min** at **65 °C** - inactivation of DNAse.

Use of Clean-Up RNA Concentration Kit (cat. # 039-25C, 039-100C)

Kit for removal and concentration of RNA samples. Elution from 15 µl. Microcolums (included with the kit) effectively bind RNA. Most contaminations flow through the microcolumns.
Elution of RNA is performed at 30 µl volume of ultrapure water and enables effective concentration.

Safety information



DANGER

Fenozol

H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye damage.
H341 Suspected of causing genetic defects.
H373 May cause damage to organs through prolonged or repeated exposure.
H411 Toxic to aquatic life with long-lasting effects.
P261 Avoid breathing dust.
P273 Avoid release to the environment.
P280 Wear protective gloves, protective clothing, eye protection, face protection.
P301+P310 If swallowed: immediately call a Poison Center or doctor/physician.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor/physician.

DANGER

Isopropanol

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

A1 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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