

Manual

Total RNA Midi

Kit for total RNA purification.

catalog #	size
032-20	20 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

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Specification

form	midicolumn
binding capacity	500 µg of RNA
sample size	<ul style="list-style-type: none"> • up to 10 ml of bacterial culture • up to 10 ml of yeast culture • up to 10 ml of blood • up to 1×10^7 of cell culture • up to 150 mg of plant or animal tissue
elution volume	from 400 µl
elution solution	ultrapure water

Contents

component	20 isolations	storage
Midicolumns	20 pcs	15–25 °C
Counterweight column	1 pcs	15–25 °C
15 ml tubes	40 pcs	15–25 °C
A1 wash solution	200 ml	15–25 °C
Fenozol	50 ml	4 °C
Isopropanol	20 ml	15–25 °C
Ultrapure water	15 ml	15–25 °C

Additional equipment and reagents

Necessary

- 15 ml sterile Falcon tubes
- Chloroform
- Centrifuge
- Heatblock or incubator set to 50 °C

Optional

- RBCL (cat. # 213-100, 213-250)
- 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

Bacterial / yeast culture

1. Centrifuge **10 ml** of overnight bacterial culture. Discard supernatants.
2. Follow point 1. of the protocol.

Cell culture

1. Centrifuge cell culture containing up to **1 x 10⁷** of cells. Discard supernatants.
2. Follow point 1. of the protocol.

Plant / animal tissue

1. Homogenize tissue sample (**50-150 mg**) in liquid nitrogen.
2. Transfer the sample to a 15 ml Falcon tube (not included).
3. Follow point 1. of the protocol.

Fresh blood (not frozen)

1. Add the appropriate amount of **RBCL** (not included) to **5-10 ml** of **blood sample**.
We recommend using 5 volumes of RBCL to 1 volume of blood sample.
2. Mix and incubate on ice for **15 min**.
Note the changing appearance of the sample during the incubation.
The initially opaque solution should turn to a completely transparent ruby-red at the incubation end.
3. Centrifuge for **5 min** at **3000 x g**. Carefully discard supernatants.
4. Follow point 1. of the protocol.

Isolation protocol

1. Add **2,4 ml** of **fenozol** and lyse cells by repetitive pipetting.

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

- at -20 °C, -80 °C up to one year
- from +4 °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.

2. Incubate sample for **5 min** at **50 °C**.

3. Add **600 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 **5 min** at **5000 RPM**.

Note: If you have an odd number of samples, please remember about counterweight columns before centrifugation.

5. Transfer the supernatant to a **new 15 ml** tube (not included).
Add **800 µl** of **isopropanol**.

6. Thoroughly mix and apply onto the midicolumn.

7. Centrifuge for **2 min** at **4000 RPM**.

8. Add **3.5 ml** of **A1** wash solution.

9. Centrifuge for **2 min** at **4000 RPM**.

10. Transfer the midicolumn to a **new 15 ml** tube (included).
Add **3.5 ml** of **A1** wash solution.

11. Centrifuge for **10 min** at **5000 RPM**.

12. Transfer the midicolumn to a **new 15 ml** tube (included).
Add **200 µl** of **ultrapure water** directly onto the minicolumn resin.

13. Keep for **3 min** at **room temp**.
Centrifuge for **2 min** at **4000 RPM**.

14. Add **200 µl** of **ultrapure water**.
15. Keep for **3 min** at **room temp**.
Centrifuge for **2 min** at **4000 RPM**.
16. Remove the midicolumn and store the tube with purified RNA at **-20 °C**, **-80 °C** until later use.

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

Total RNA Midi 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 **400 µl** of **RNA eluate** add:

 4 µl of **DNase** (10 U/µl)
 40 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. Microcolumns (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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