A&A BIOTECHNOLOGY
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Manual

Total RNA Mini Concentrator

Kit for total RNA purification.

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

Spis treści

Specification

form	microcolumn
binding capacity	10 µg of RNA
sample size	• up to 500 µl of bacterial culture • up to 500 µl of yeast culture • up to 1 ml of blood • up to 1 x 10^5 of cell culture • up to 10 mg of plant or animal tissue • Nematode worm
elution volume	from 15 µl
elution solution	ultrapure water

Contents

component	25 isolations	100 isolations	storage
Microcolumns	25 pcs	100 pcs	15–25 °C
1.5 ml tubes	25 pcs	100 pcs	15–25 °C
2 ml tubes	50 pcs	200 pcs	15–25 °C
A1 wash solution	50 ml	200 ml	15–25 °C
Fenozol	25 ml	100 ml	2–8 °C
Isopropanol	13 ml	30 ml	15–25 °C
Ultrapure water	1.5 ml	2 x 1.5 ml	-20–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Chloroform
- Microcentrifuge
- 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 **100-500 µl** of overnight bacterial culture. Discard supernatants.
2. Follow point 1. of the protocol.

Cell culture

1. Centrifuge cell culture containing up to **1 x 10^5-5 x 10^5** of cells. Discard supernatants.
2. Follow point 1. of the protocol.

Plant / animal tissue

1. Homogenize tissue sample **(1-10 mg)** in liquid nitrogen.
2. Transfer the sample to 1.5 ml Eppendorf tube (not included).
3. Follow point 1. of the protocol.

Fresh blood (not frozen)

1. Add **the appropriate amount** of **RBCL** (not included) to **maximum 1 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 **10 min** at **3000 x g.** Carefully discard supernatants.
4. Follow point 1. of the protocol.

Fresh or frozen Nematode worm

1. Place individual Nematode worm on a sterile surface (e.g. small Petri dish)and cut the worm into 3-4 pieces with a sterile blade. Transfer all fragments to 1.5 ml Eppendorf tube (not included).
2. Follow point 1. of the protocol.

Isolation protocol

1. Add **800 µl** 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 +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.

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).
   Add **250 µl** of **isopropanol**.

6. Thoroughly mix and apply onto the microcolumn. Close the tube with the cap.

7. Centrifuge for **1 min** at **12 000 RPM**.

8. Transfer the microcolumn to **a new** 2 ml tube (included).
   Add **700 µl** of **A1** wash solution. Close the tube with the cap.

9. Centrifuge for **1 min** at **12 000 RPM**.

10. Remove the microcolumn from the tube and discard the filtrate.
    Place the microcolumn into the same tube. Add **700 µl** of **A1** wash solution.
    Close the tube with the cap.

11. Centrifuge for **1 min** at **12 000 RPM**.

12. Transfer the microcolumn to **a new** 2 ml tube (included).
    Add **200 µl** of **A1** wash solution. Close the tube with the cap.

13. Centrifuge for **2 min** at **12 000 RPM**.

14. Transfer the dry microcolumn to **a new** 1.5 ml elution tube (included).
Add **15-20 µl** of **ultrapure water** directly onto the microcolumn resin.
Close the tube with the cap.

15. Keep for **3 min** at **room temp.**
Centrifuge for **1 min** at **12 000 RPM**.

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

Elution tube has a long, elastic cap connector. Start closing the tube by careful pressing the cap on the connector side.
An opening „click" sound confirms proper closure. Different ways of closing may cause opening of the tube during storage.

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

Total RNA Mini Concentrator 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 20 µl** of **RNA eluate** add:

   0.2 µl of **DNAse** (10 U/µl)
   2 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.

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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