A&A BIOTECHNOLOGY
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Manual

Micro RNA Concentrator

Kit for microRNA purification. Chloroform free procedure.

catalog #	size
035-25C	25 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	microcolumn
binding capacity	10 µg of RNA
sample size	• up to 3 ml of bacterial or yeast culture • up to 2 ml of blood • up to 1 x 10^6 of cell culture • up to 50 mg of plant or animal tissue
elution volume	from 15 µl
elution solution	ultrapure water

Contents

component	25 isolations	storage
Minicolumns	25 pcs	15–25 °C
Microcolumns	25 pcs	15–25 °C
2 ml tubes	50 pcs	15–25 °C
1.5 ml tubes	25 pcs	15–25 °C
A1 wash solution	50 ml	15–25 °C
Fenozol Plus	12 ml	4 °C
Isopropanol	20 ml	15–25 °C
Ultrapure water	8 ml	15–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Microcentrifuge
- Heatblock or incubator set to 50 °C

Optional

- A1 wash solution, ultrapure water, RBCL
- 1.5 ml, 2 ml sterile Eppendorf tubes

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 1-3 ml of overnight bacterial culture / yeast culture. Discard the supernatant.
2. Follow point 1. of the protocol for low RNA molecular weight.

Cell culture

1. Centrifuge cell culture containing up to 1 x $10^6$of cells. Discard the supernatant.
2. Follow point 1. of the protocol for low RNA molecular weight.

Plant / animal tissue

1. Homogenize tissue sample **(20-50 mg)** in liquid nitrogen.
2. Transfer the sample into 1.5 ml Eppendorf tube (not included).
3. Follow point 1. of the protocol for low RNA molecular weight.

Fresh blood (not frozen)

1. Add the equivalent of five volumes of **RBCL** (not included) to **1-2 ml** 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 supernatant.
4. Follow point 1. of the protocol for low RNA molecular weight.

Serum or plasma

1. Transfer 100 µl of serum or plasma into 1.5 ml Eppendorf tube (not included).
2. Follow point 1. of the protocol for low RNA molecular weight.

Isolation protocol for low RNA molecular weight

1. Add **400 µl** of **fenozol Plus** and lyse cells by repetitive pipetting.

   Fenozol Plus deactivates endogenous RNAses. Sample suspended in fenozol Plus 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 Plus contains phenol. Avoid contact with skin. Wear suitable protective gloves.

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

3. Add **150 µl** of **ultrapure water**.

4. Vortex intensely for **15 s**. Keep the sample for **3 min** at **room temp**.

5. Centrifuge the sample for **10 min** at **10 000-12 000 RPM**.

6. Transfer the supernatant to **a new** 1.5 ml tube (not included). Add **150 µl** of isopropanol.
   In case of isolation from serum or plasma add 180 µl of isopropanol.

7. Thoroughly mix and apply onto the **minicolumn**.
   Centrifuge for **1 min** at **10 000-12 000 RPM**.

   High molecular RNA is bounded to minicolumn, while low molecular RNA is not bounded and is present in the tube.
   In order to recover high molecular weight RNA from minicolumn, proceed to isolation protocol for high molecular weight RNA.

8. Remove the **minicolumn** from the tube. Add **400 µl** of **isopropanol** to the filtrate.
   In case of isolation from serum or plasma add 500 µl of isopropanol.

9. Mix by pipetting. Apply **600 µl** of **mixture** onto a new **microcolumn**. Close the tube with the cap.

   Note: The maximum loading volume of the microcolumn is 600 µl.

10. Centrifuge for **1 min** at **10 000-12 000 RPM**.

11. Remove the **microcolumn** from the tube and discard the filtrate.
    Place the **microcolumn** into the same tube.
    Apply the remaining part of the mixtures onto the **microcolumn**. Close the tube with the cap.

12. Centrifuge for **1 min** at **10 000-12 000 RPM**.

13. Transfer the **microcolumn** to **a new** 2 ml tube with cap (included). Add **300 µl** of **A1** wash solution. Close the tube with the cap.

14. Centrifuge for **1 min** at **10 000-12 000 RPM**.

15. Add **300 µl** of **A1** wash solution. Close the tube with the cap.

16. Centrifuge for **1 min** at **10 000-12 000 RPM**.

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

18. Centrifuge for **2 min** at **10 000-12 000 RPM**.

19. Transfer the dry minicolumn to **a new** 1.5 ml elution tube (not included). Add **15-20 µl** of **ultrapure water** directly onto the minicolumn resin. Close the tube with the cap.

20. Keep for **3 min** at **room temp.**

21. Centrifuge for **1 min** at **10 000-12 000 RPM**.

22. Remove the **microcolumn**, close the tube 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.

Isolation protocol for high RNA molecular weight

A1 wash solution, ultrapure water, 1.5 ml, 2 ml tubes should be ordered separately.

1. Transfer the minicolumn to **a new** 2 ml tube (not included). Add **700 µl** of **A1** wash solution.

2. Centrifuge for **1 min** at **10 000-12 000 RPM**.

3. Remove the minicolumn from the tube and discard the filtrate. Place the minicolumn into the same tube. Add **700 µl** of **A1** wash solution.

4. Centrifuge for **1 min** at **10 000-12 000 RPM**.

5. Remove the minicolumn from the tube and discard the filtrate.
Place the minicolumn into the same tube. Add **200 µl** of **A1** wash solution.

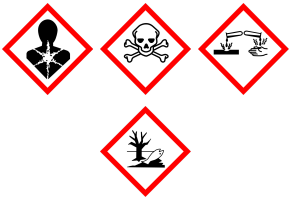
6. Centrifuge for **2 min** at **10 000-12 000 RPM**.

7. 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.

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

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

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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