A&A BIOTECHNOLOGY
innovating life science

Total RNA Mini Plus

Kit for total RNA isolation. Chloroform free procedure.
version 0920

25 isolations, 100 isolations

Cat. # 036-25, 036-100

The binding capacity of the RNA purification column is 100 µg of RNA.

For R&D use only.

Kit Contents

Component	25 isolations	100 isolations	Store at
Minicolumns for RNA isolation	25 pcs	100 pcs	Room Temp.
2 ml tubes	50 pcs	200 pcs	Room Temp.
A1 wash solution	50 ml	200 ml	Room Temp.
Fenozol Plus	15 ml	50 ml	+4 °C
Isopropanol	15 ml	50 ml	Room Temp.
Sterile water (nuclease free, DEPC treated)	8 ml	30 ml	from -20 °C to +20 °C

Equipment and materials necessary for RNA isolation that are not included in kit

1. Material for RNA isolation
2. RBCL – buffer for red blood cells lysis for blood samples only (cat. # 213-100, 213-250)
3. 1.5 ml sterile Eppendorf tube
4. Benchtop microcentrifuge
5. Heatblock or incubator set to 50 °C

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Material preparation

A. Bacteria culture

1. Centrifuge 1–3 ml of overnight bacterial culture.
   Discard the supernatant.
2. Proceed to Isolation protocol, point 1.

B. Yeast

1. Centrifuge 1 ml of yeast culture.
   Discard the supernatant.
2. Proceed to Isolation protocol, point 1.

C. Cell culture

1. Centrifuge tissue culture containing up to $1x10^6$ cells.
   Discard the supernatant.
2. Proceed to Isolation protocol, point 1.

D. Tissues

1. Homogenize tissue samples (20–50 mg) in liquid nitrogen.
2. Transfer the sample into a 1.5 ml Eppendorf tube (not included) .
3. Proceed to Isolation protocol, point 1.

E. Plant tissues

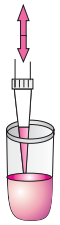
1. Homogenize tissue samples (20–50 mg) in liquid nitrogen.
2. Transfer the sample into a 1.5 ml Eppendorf tube (not included).
3. Proceed to Isolation protocol, point 1.

F. Fresh blood (1–2 ml) (not for frozen blood samples)

1. Add (the equivalent of five volumes of RBCL – buffer for red blood cells lysis (not included, cat. # 213–100, 213–250) to blood sample.
2. Mix the contents 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 end of incubation.
3. Centrifuge the sample for 10 min at 3000 x g.
   Carefully discard the supernatant.
4. Proceed to Isolation protocol, point 1.

Isolation protocol

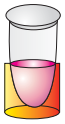
1. Add 400 µl of Fenozol Plus and lyse cells by repeated pipetting.



Fenozol Plus inactivates endogenious 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

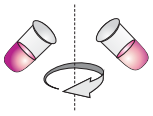
Attention: Fenozol Plus contains phenol. Avoid contact with skin. Wear suitable protective gloves.

2. Incubate the samples for 5 min at 50 °C.



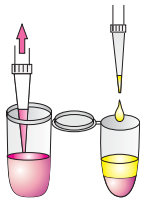
3. Add 150 µl of sterile water. Vortex intensely for 15 s. Incubate the samples for 5 min at room temp.

4. Centrifuge the samples for 15 min at 10 000–12 000 RPM.

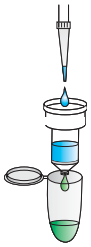


During the centrifugation step the DNA and proteins are collected at the bottom tube while RNA stay dissolved in the supernatant.

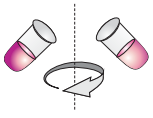
5. Transfer 400 µl of the supernatant to new 1.5 ml Eppendorf tubes (not included).
Add 400 µl of Isopropanol.



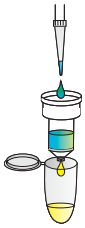
6. Throughly mix and apply onto the minicolumns.



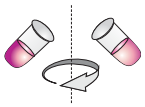
7. Centrifuge for 1 min at 10 000–12 000 RPM.



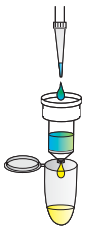
8. Transfer the minicolumns to new 2 ml tubes (included).
Add 700 µl of A1 wash solution.



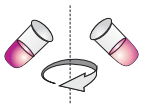
9. Centrifuge for 1 min at 10 000–12 000 RPM.



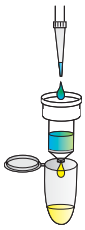
10. Transfer the minicolumns to new 2 ml tubes (included).
Add 700 µl of A1 wash solution.



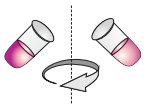
11. Centrifuge for 1 min at 10 000–12 000 RPM.



12. Discard the flow through from the 2 ml tubes and re-attach the minicolumns.
Add 300 µl of A1 wash solution.

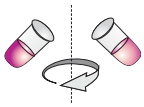


13. Centrifuge for 2 min at 10 000–12 000 RPM.



14. Transfer the dry minicolumns into new 1.5 ml elution tubes (not included).
Add 50–100 µl of sterile water.

15. Incubate for 2 min at room temp.
Centrifuge for 1 min at 10 000–12 000 RPM.



16. Remove the minicolumns, close the elution tubes.
Store the purified RNA samples at –20 °C or –80 °C.

Summary Protocol

prepared sample + 400 µl Fenozol Plus

i. mix
ii. incubate at 50 °C – 5 min

sample with Fenozol Plus + 150 µl sterile water

i. mix
ii. incubate at room temp. – 5 min
iii. centrifuge 10 000–12 000 RPM – 15 min

supernatant → new 1.5 ml tube ← 400 µl Isopropanol

i. mix
ii. load onto minicolumn
iii. centrifuge 10 000–12 000 RPM – 1 min

new 2 ml tube ← minicolumn ← 700 µl A1 wash solution

i. centrifuge 10 000–12 000 RPM – 1 min

new 2 ml tube ← minicolumn ← 700 µl A1 wash solution

i. centrifuge 10 000–12 000 RPM – 1 min
ii. discard the flow through from the 2 ml tube and re-attach the minicolumn

300 µl A1 wash solution → minicolumn

i. centrifuge 10 000–12 000 RPM – 2 min

1.5 ml elution tube ← minicolumn ← 50–100 µl sterile water

i. incubate at room temp. – 2 min
i. centrifuge 10 000–12 000 RPM – 1 min

50–100 µl RNA

Ready to use
Store the purified RNA sample at –20 °C or –80 °C

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

Total RNA Mini Plus 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 using Clean-Up RNA Concentrator Kit (cat. # 039-25C, 039-100C)
Microcolums (included in the kit) effectively bind RNA. Most contaminations flow through the microcolumns.
Elution of RNA is performed at 30 µl volume of sterile water and enables effective concentration.

Ordering Information

Product	Quantity	Cat.#
RBCL buffer for red blood cells lysis	100 ml	213-100
	250 ml	213-250
Fenozol Plus	50 ml	203-50P
Isopropanol	50 ml	208-50
Sterile water (nuclease free, DEPC treated)	5 x 1.5 ml	003-075
	5 x 5 ml	003-25
	500 ml	003-500
Clean-Up RNA Concentrator	25 isolations	039-25C
	100 isolations	039-100C

Safety information

DANGER

Fenozol Plus

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 protect 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

A1 wash solution

H225 Highly flammable liquid and vapour.
H319 Cuses serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
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

Isopropanol

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