A&A BIOTECHNOLOGY
innovating life science

Manual

MagnifiQ™ 1 Total RNA Plus instant kit

Kit for automated, magnetic isolation of total RNA in the strip format. Contains ready-to-use, reagent-filled strips and all necessary consumables. The strip format enables isolation of a single sample per purification run.

catalog #	size	compatible devices *
614A-1V-32	32 isolations	Auto-Pure Mini Auto-Pure S32
614A-1V-160	160 isolations	Auto-Pure Mini Auto-Pure S32

*** Compatible devices**
The kit has been tested with specific Allsheng brand isolation devices. This does not preclude it from working with other devices. If your device is not listed, please contact us at info@aabiot.com.

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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Advantages

- Requires only a few minutes of manual work while adding the samples. The rest of the procedure is carried out by automatic extraction device.
- It does not require the initial preparation of buffers. Just add samples to the plate and after about 30 min you get extracted material.
- Enables RNA isolation from single sample.

Sample type

	sample size
Bacteria G+ (cultures)	up to 2 x 10^8
Bacteria G- (cultures)	up to 2 x 10^8
Cell cultures	up to 2 x 10^6
Fresh blood (not frozen)	up to 2.5 ml
Fresh blood (preserved in PAXgene Blood RNA Tubes)	up to 2.5 ml
Solid tissue	20 - 50 mg

Specification

protocol time	~ 30 min.
elution volume	100 µl [1]
elution solution	Tris buffer
binding capacity	30 µg RNA
downstream applications	reverse transcription RT-qPCR, transcriptome sequencing

[1] The elution volume prepared on the strip is 100 µl. To obtain a smaller elution volume, subtract the appropriate amount of elution solution from the well 6 on the XS-R strip. Attention! Do not reduce the elution volume below 50 µl.

Description

MagnifiQ™ 1 Total RNA Plus instant kit is designed for total RNA isolation from various types of biological materials. The isolated material is perfect for further analyzes and tests by RT-PCR methods and for sequencing.

The **MagnifiQ™** product series is based on the automated isolation of nucleic acids with use of magnetic beads. This method significantly shortens working time and reduces risk of mistake in comparison to manual methods.

Contents

	620A-1V-32		620A-1V-160		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
XS-R - extraction strip	4 x 8 pcs	K-S1V22XR	20 x 8 pcs	K-S1V22XR	15–25 °C
Fenozol Plus	20 ml	K-FENP-20	90 ml	K-FENP-90	2–8 °C
ultrapure water	8 ml	K-WUP-8	40 ml	K-WUP-40	-20–25 °C
tip comb 8	8 x 2 pcs	K-C8U-2	40 x 2 pcs	K-C8U-2	15–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml Eppendorf tubes (sample lysis)
- pipette
- pipette tips
- vortex
- thermoblock
- centrifuge
- centrifuge for swing-out rotor (capable of centrifuging PAXgene Blood RNA tubes - round bottom adaptors or 15 ml Falcon type alternatives)

Material preparation

Bacteria G+ (cultures)

Additional reagents you will need:

- **BacBreaker** bacteria lysis enzyme mix (20 µl per sample), cat # BACB-15A

Option:

- **Lysostaphin** (20 µl per sample), cat # 1007-3; For *Staphylococcus spp.* we recommend using lysostaphin.

1. Transfer the sample of the bacterial culture to the 1.5 ml Eppendorf tube (not included). Centrifuge for **3 min** at **10 000 RPM**. Discard the supernatant.
2. Suspend the bacterial pellet in **50 µl** of **ultrapure water**.
3. Add **20 µl** of **BacBreaker** enzyme mix.

   Attention. For lysis of *Staphylococcus* bacteria, add **20 µl** of **lysostaphin**.
4. Vortex the sample for **10 s** and incubate for **10 min** at **37 °C**.
5. Add **500 µl** of **Fenozol Plus.**
6. Close the tube and mix whole content by inverting the tube. Incubate for **5 min** at **50 °C**.
7. Add **170 µl** of **ultrapure water**. Close the tube and mix whole content by inverting the tube.

   Note. Adding water results in DNA precipitation.
8. Centrifuge the sample for **10 min** at **12 000 RPM**.
9. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

   Follow point 1. of the protocol.

Bacteria G- (cultures)

1. Transfer the sample of bacterial culture to 1.5 ml Eppendorf tube (not included).
Centrifuge for **3 min** at **10 000 RPM**. Discard the supernatant.

2. Add **500 µl** of **Fenozol Plus.**

3. Close the tube and mix whole content by inverting the tube.
Incubate for **5 min** at **50 °C**.

4. Add **170 µl** of **ultrapure water**.
Close the tube and mix whole content by inverting the tube.

Note. Adding water results in DNA precipitation.

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

6. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

Follow point 1. of the protocol.

Cell cultures

1. Transfer the sample of the cell culture to 1.5 ml Eppendorf tube (not included).
Centrifuge for **3 min** at **10 000 RPM**. Discard the supernatant.

2. Add **500 µl** of **Fenozol Plus.**

3. Close the tube and mix whole content by inverting the tube.
Incubate for **5 min** at **50 °C**.

4. Add **170 µl** of **ultrapure water**.
Close the tube and mix whole content by inverting the tube.

Note. Adding water results in DNA precipitation.

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

6. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

   Follow point 1. of the protocol.

Fresh blood (not frozen)

Additional reagents you will need:

- **RBCL** (max. 5 ml per sample), cat # 213-100

1. Add **the appropriate amount** of **RBCL** to **2.5 ml** of **blood sample**.

   Attention. We recommend using 5 volumes of RBCL to 1 volume of blood sample.

2. Mix and incubate on ice for **15 min**.

   Note. 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. Add **500 µl** of **Fenozol Plus.**

5. Close the tube and mix whole content by inverting the tube.
   Incubate for **5 min** at **50 °C**.

6. Add **170 µl** of **ultrapure water**.
   Close the tube and mix whole content by inverting the tube.

   Note. Adding water results in DNA precipitation.

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

8. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

   Follow point 1. of the protocol.

Fresh blood (preserved in PAXgene Blood RNA Tubes*)

Purification of total RNA from 2.5 ml human whole blood collected in a PAXgene Blood RNA Tube accordingly to manufacturer instructions in the PAXgene Blood RNA Tubes Handbook.

Additional reagents you will need:

- **ultrapure water** (sterile, nuclease-free, 4 ml per sample), cat # 005-100

*Paxgene Blood RNA is a trademark owned by Qiagen.
** BD Hemogard is a trademark owned by BD (Becton Dickingson)

Before starting the isolation process, blood samples stored in PAXgene tubes must be kept at room temperature for at least 2 hours to ensure complete lysis of blood cells. If the PAXgene Blood RNA Tubes have been stored at 2–8 °C, –20°C, or –70 °C after blood collection, they should first be equilibrated to room temperature and then stored at room temperature for 2 hours before starting the isolation procedure.

1. Centrifuge the PAXgene Blood RNA Tubes for **10 min** at **3000 - 5000 x g** using a swing-out rotor.

   Note. The rotor must contain tube adapters for round-bottom tubes. If other types of tube adapter are used, transfer the whole content with a sterile tip to a suitable tube and proceed centrifugation.

2. Carefully discard the supernatant.
   Add **4 ml** of **ultrapure water** to the pellet and close the tube using a fresh secondary BD Hemogard closure (supplied with the PAXgene Blood RNA Tubes).

   Note. When removing the supernatant, be careful not to disturb the pellet and dry the tube rim with a clean paper towel.

3. Vortex until the pellet is visibly dissolved and centrifuge for **10 min** at **3000 - 5000 x g** using a swing-out rotor.

   Note. The presence of residual undissolved solids after vortexing but before centrifugation will not affect the isolation procedure.

4. Discard the supernatant.

   Note. Incomplete removal of the supernatant will inhibit lysis and dilute the lysate, and therefore affect the conditions for binding RNA along the purification process.

5. Add **500 µl** of **Fenozol Plus** and to mix the sample and resuspend the pellet, transfer the whole mixture to 1.5 ml Eppendorf tube (not included).

6. Close the tube and mix whole content by inverting the tube.
   Incubate for **5 min** at **50 °C**.

7. Add **170 µl** of **ultrapure water**.
   Close the tube and mix whole content by inverting the tube.

   Note. Adding water results in DNA precipitation.

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

9. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

   Follow point 1. of the protocol.

Solid tissue

1. Homogenize tissue sample in liquid nitrogen.
   Transfer the sample of tissue to 1.5 ml Eppendorf tube (not included).

   Note. For soft tissues, use mechanical lysis. Transfer the sample to a tube containing zirconia beads (A&A Biotechnology cat. # 106-50Z). Place the tube in the Beadbeater and perform the shaking process.

2. Add **500 µl** of **Fenozol Plus.**

3. Close the tube and mix whole content by inverting the tube.
   Incubate for **5 min** at **50 °C**.

4. Add **170 µl** of **ultrapure water**.
   Close the tube and mix whole content by inverting the tube.

   Note. Adding water results in DNA precipitation.

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

6. **Attention.** In the isolation protocol, use **600 µl** of supernatant as the sample. Take the filtrate starting from the top so that the precipitate is not carried over to the next purification step. The step is important for RNA purity.

   Follow point 1. of the protocol.

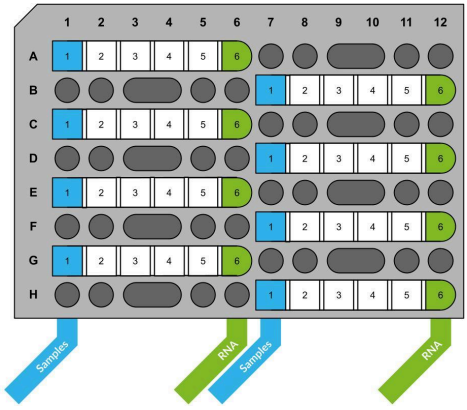
Protocol

Protocol files

device	protocol name	protocol file	installation
Auto-Pure Mini	MQ-RNA-MI	aabiot.com/protocols/magnifiq/MI/MQ-RNA-MI.txt	1. Create folder "items" on a USB drive and copy the protocol file to it. 2. Insert the USB drive into a USB slot in the device. 3. On a device screen, go to Settings > System > Transfer >Import. 4. Select the protocol and tap "Import".
Auto-Pure Mini (QR code)	MQ-RNA-MI		1. On a device screen, go to Run > ☰ > ⌜⌝ 2. Scan the QR code with the device's scanner.
Auto-Pure S32	MQ_RNA_S32	aabiot.com/protocols/magnifiq/S32/MQ_RNA_S32.txt	1. Create folder "im_export_protocols" on a USB drive and copy the protocol file to it. 2. Insert the USB drive into a USB slot in the device. 3. On a device screen, go to Protocols >Import. 4. Select the protocol and tap "Import".

Extraction protocol

1. Place **XS-R** strips in the rack.



2. Remove the foil from the **XS-R** strips.

3. Add **600 µl** of samples to the well **1** (first from the left) on the **XS-R** strip.

   Note. The wells are numbered on the side of the strip.

   Attention: If no pellet is visible at the bottom of the tube, take the filtrate starting from the top so as not to mix the whole after centrifugation. This step is intended to separate the fraction containing DNA.

4. Place the rack in the extraction device.

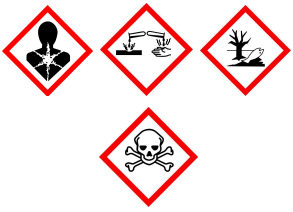
5. Place the appropriate number of **tip combs 8** in the extraction device.

6. Run the protocol on your device.

7. After the program is over, remove the combs and then remove the rack from the extraction device and transfer RNA located in well **6** (first from the right) on the **XS-R** strip into sterile tubes (not included).

   Note. Store extracted material at -80 °C.

Safety information



DANGER

Fenozol Plus

HH301+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

XS-R - extraction strip

H225 Highly flammable liquid and vapor.
H302+H312+H332 Harmful if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye damage.
H412 Harmful to aquatic life with long lasting effects.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P273 Avoid release to the environment.
P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P301+P312+P330 If swallowed: Call a poison center/doctor/ if you feel unwell.
P303+P361+P353 If on skin (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P304+P340 If inhaled: Remove person to fresh air and keep comfortable for breathing.
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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