A&A BIOTECHNOLOGY
innovating life science

Manual

MagnifiQ™ 16 Total RNA Plus instant kit

Kit for automated, magnetic isolation of total RNA in the 16 samples per plate format. Contains ready-to-use, reagent-filled plates and all necessary consumables.

catalog #	size	compatible devices *
614A-16U-64	64 isolations	Auto-Pure 32A
614A-16V-64	64 isolations	Auto-Pure Mini
614A-16U-256	256 isolations	Auto-Pure 32A
614A-16V-256	256 isolations	Auto-Pure Mini

*** 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

- MagnifiQ™ 16 Total RNA Plus instant kit does not require initial preparation of buffers. Just add samples to the plate and get extracted material within approximately half an hour.
- It enables RNA isolation from different types of material at the same time with universal kit and automated extraction programme.

Sample type

	sample size
Bacteria G+ (cultures)	up to 2 x 10^8
Bacteria G- (cultures)	up 2 x 10^8
Cell cultures	up 2 x 10^6
Fresh blood (not frozen)	up 1 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-PCR, transcriptome sequencing

[1] The elution volume prepared on the plate is 100 µl. To obtain a smaller elution volume, subtract the appropriate amount of elution solution from the wells of columns 6 and 12 on the XP-R plate. Attention! Do not reduce the elution volume below 50 µl.

Description

MagnifiQ™ 16 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

	614A-16U-64		614A-16U-256		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
XP-R - extraction plate	4 pcs	K-P96U22XR	16 pcs	K-P96U22XR	15–25 °C
Fenozol Plus	35 ml	K-FENP-35	140 ml	K-FENP-140	4–8 °C
ultrapure water	15 ml	K-WUP-15	60 ml	K-WUP-60	-20–25 °C
tip comb 8	4 x 2 pcs	K-C8U-2	16 x 2 pcs	K-C8U-2	15–25 °C
protective film	4 pcs	K-MQF-4	16 pcs	K-MQF-16	15–25 °C

	614A-16V-64		614A-16V-256		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
XP-R - extraction plate	4 pcs	K-P96V22XR	16 pcs	K-P96V22XR	15–25 °C
Fenozol Plus	35 ml	K-FENP-35	140 ml	K-FENP-140	4–8 °C
ultrapure water	15 ml	K-WUP-15	60 ml	K-WUP-60	-20–25 °C
tip comb 8	4 x 2 pcs	K-C8U-2	16 x 2 pcs	K-C8U-2	15–25 °C
protective film	4 pcs	K-MQF-4	16 pcs	K-MQF-16	15–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml tubes (sample lysis)
- automated pipette
- pipette tips
- centrifuge
- vortex
- thermoblock

Optional

- 2.2 ml plates (sample lysis)
- swing-out rotor centrifuge for 96-well plates
- protective film (lysis in a 96 deep-well plate)

Important notes

The following material preparation protocols apply to the procedure carried out in 1.5 ml tubes. If the material preparation is to be carried out in a 96 deep-well plate see the Additional information.

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 bacterial culture sample containing **2 x 10^8** bacteria to the 1.5 ml 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 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. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.
7. Add **170 µl** of **ultrapure water**.
   Vortex the sample for **10 s.**

   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 the 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 bacterial culture sample containing **2 x 10^8** bacteria 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. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.
4. Add **170 µl** of **ultrapure water**.
   Vortex the sample for **10 s.**

   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 the 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 cell culture sample containing **2 x 10^6** cells 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. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.
4. Add **170 µl** of **ultrapure water**.
   Vortex the sample for **10 s.**

   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 the 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 **1 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. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.

6. Add **170 µl** of **ultrapure water**.
   Vortex the sample for **10 s.**

   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 the 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. Vortex the sample for **10 s** and incubate for **5 min** at **50 °C**.

4. Add **170 µl** of **ultrapure water**.
Vortex the sample for **10 s.**

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 the 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

1. Carefully centrifuge the **XP-R** plate for **1 min** at **2000 RPM.**

2. Remove the foil from the **XP-R** plate.

3. Add **600 µl** of samples to the wells in columns **1** and **7** of the **XP-R** plate.

   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 one or two **XP-R** plates in the extraction device.

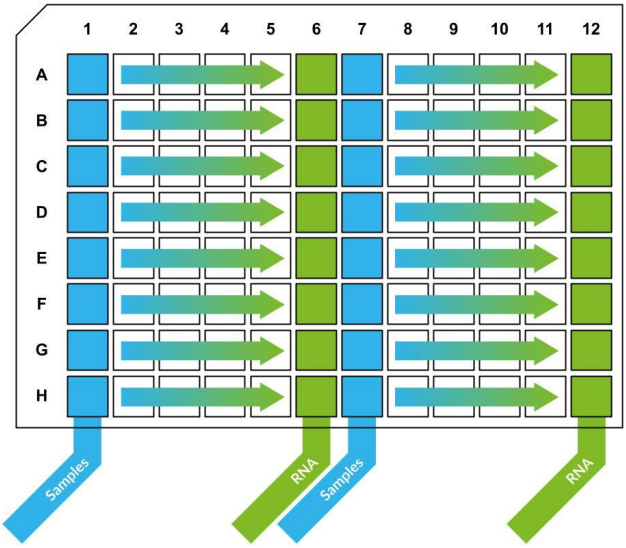
5. Place the appropriate number of t**ip combs 8** in the extraction device.

6. Run the program according to the table below:

Step	Well	Name	Mix Time (min)	Magnet (sec)	Wait Time (min)	Volume (µl)	Mix Speed (1-10)	Temp. (°C)
1	1	MIX	5.0	0	0.0	900	4	OFF
2	5	BEADS	0.5	30	0.0	600	5	OFF
3	1	BIND	5.0	60	0.0	900	4	OFF
4	2	WASH1	2.0	30	0.0	600	6	OFF
5	3	WASH2	1.0	30	0.0	600	6	OFF
6	4	WASH3	1.0	30	5.0	600	6	OFF
7	6	ELUTION	6.0	60	0.0	100	8	50
8	3	DROP	1.0	0	0.0	600	5	OFF

7. After the program is over, remove the combs and then remove **XP-R plate** from the extraction device and seal it with **protective film**. The extracted RNA is located in columns **6** and **12**.

   Note. For longer storage of extracted material, transfer it from the plate to appropriate tubes and store at -80 °C.



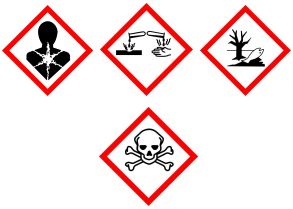
Additional information

Preparation of material in a 96 deep-well plate

Lysis of the material in a 96 deep-well plate should be carried out according to the respective procedure for 1.5 ml Eppendorf tubes in the Material Preparation section. The following changes should be made:

- Incubation parameters
  Increase the incubation temperature by **5 °C** and extend the time by **10 min** with a minimum of **1000 RPM** continuous shaking speed.
- Centrifugation parameters
  Centrifuge the plate for **5 min** at **1 000 x g**.

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

XP-R - extraction plate

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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