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Magnetic total RNA isolation kit

Cat. No. NAmagp100

Manual and automatic methods for RNA isolation

Important!

We are regularly improving the protocol for working with the kit. Please use the protocol included with the kit. The latest version of the protocol is available on the website of Biolabmix LLC (www.biolabmix.ru).

The kit is intended for research purposes only.

The protocol was updated on February 2024.

Description

The kit is designed for total RNA isolation and purification swabs and scrapes of epithelial cells, viruses. The method of RNA isolation is based on the selective binding of nucleic acids from a lysed sample on magnetic particles formed with iron and silica oxides, followed by washing and elution of the purified RNA. The integrity of RNA is preserved in the process of isolation.

It is possible to extract RNA both manually by using a magnetic stand and automatically by using the automatic nucleic acid purification systems **Auto-Pure96 (Allsheng)** and **KingFisherFlex (ThermoScientific).**

Important! Isolated RNA contains admixture of DNA. DNase treatment is required for subsequent use of RNA sample in applications sensitive to the presence of DNA, for example, PCR.

Important! The difference between Magnetic total RNA isolation kits Cat. No. NAmagp100 and Cat. No. MRP100 is that the lysis buffers (LB) and washing (WB) in position Cat. No. MRP100 contains isopropanol. When using position Cat. No. NAmagp100 it is necessary to use ethanol (for more details, see the sections "**Before starting the procedures**" and "**RNA isolation protocol**").

RNA isolation time by the automatic systems Auto-Pure96 (Allsheng) and KingFisherFlex (ThermoScientific) is 20 minutes.

Kit contents

Cat. No.	NAmagp100 100 preps
Lysis buffer LB	45 ml
Wash buffer WB (concentrate)	22 ml
Elution buffer EB	15 ml
Magnetic particles M	1.2 ml

Safety Information

Caution! Lysis LB buffer contains chaotropic salt solution, which is irritating and toxic. Buffers are toxic in contact with skin and insides, causing burns. You must follow the rules of general and personal safety when working with the kit.
In case of a contact with skin, wash immediately with plenty of water and detergents. Visit a doctor if necessary.
Warning! When working with biological fluids, wear disposable gloves, since material may potentially be infected and capable of storing or transmitting HIV, hepatitis virus or any other infection for a long time. All used materials should be disinfected and disposed in accordance with local requirements.

Operation

Components: LB, WB, EB and M are stable after opening throughout the entire shelf life if stored in appropriate conditions and sufficiently sealed. Storage conditions are indicated on the kit and reagents labels.
Caution! Do not store the mixture of lysis buffer LB and magnetic particles M.

Operation conditions

Ambient temperature from +15 to +25 °C;
Relative air humidity less than 80 %;
Atmosphere pressure 630 – 800 mmHg.

Equipment and reagents to be supplied by user

Manual protocol

- Dry block incubator capable to reach temperature 65 °C;
- Magnetic rack for 1.5-2 ml microtubes;
- Vortex;
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes;
- Ethanol, 96-100%.

Automatic protocol

- 96 deepwell plate, V-shaped bottom, 2 ml wells, 3 pcs;
- 96 tip comb for deep-well magnets, 1 pcs;
- Ethanol, 96-100 %.

Before starting the procedures

Preparing the lysing and binding mixture

When using an automatic nucleic acid purification system, prepare a mixture of lysis buffer LB, ethanol (96-100%), and magnetic particles M. Prepare the required volume of this mixture based on the number of samples.

Note: It is recommended to increase the desired volume by 10% when working with multiply samples.

- **1 prep.** Mix 300 µl of lysis buffer (LB), 400 µl of ethanol (96-100%), 10 µl of magnetic particles suspension to obtain the solution with total volume 710 µl.
- **100 preps (+10%).** Mix 33 ml of lysis buffer (LB), 44 ml of ethanol (96-100%), and 1.1 ml of magnetic particles suspension to obtain the solution with total volume 78.1 ml.

Preparing the WB buffer

- **1 prep, 500 µl WB.** Add 400 µl of ethanol (96-100%) to 100 µl of WB (concentrate).
- **100 preps.** Add 88 ml of ethanol (96-100%) to 22 ml of WB (concentrate).

It is recommended to add ethanol to the aliquots of the WB, since ethanol may partially evaporate when storing the buffer for several months.

RNA isolation protocol. Manual protocol

RNA isolation is carried out at room temperature (15-25 °C).

1) Preparing and lysing the samples

1. Take a 100 µl aliquot of saline or transport medium after incubation of a cotton swabs from nose or mouth to a clear 1.5 ml tube.
2. Add 400 µl LB.
3. Mix thoroughly by pipetting, avoid foaming.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at 50 °C.

2) RNA binding to magnetic particles

1. Mix magnetic particles suspension thoroughly by stirring manually or by vortex to obtain homogeneous suspension.
2. Add equal volume of ethanol to lysate. For example, if sample volume is 100 µl, and LB volume is 400 µl, 500 µl of ethanol is needed. Mix thoroughly to obtain homogeneous suspension.
3. Add 10 µl of magnetic particles suspension to the sample and immediately mix by pipetting or vortex to obtain homogeneous suspension.
4. Incubate for 10 min.
5. Place the tube with sample to magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

6. Discard the supernatant while the tube in magnetic rack. Don't disturb magnetic particles at the tube wall.

3) Magnetic particles washing

1. Add 500 µl of WB to the tube. Mix thoroughly by pipetting or vortexing to obtain homogeneous suspension.

Note: Ensure that ethanol was added to the WB.

2. Place the tube in magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

3. Discard the supernatant while the tube in magnetic rack. Don't disturb magnetic particles at the tube wall.
4. Repeat steps 1-4 using WB.
5. Dry the tube with magnetic particles in air at 15-25 °C for 5-15 minutes or until completely dry.

4) RNA elution

1. Add 50-100 µl of EB. Mix thoroughly by pipetting or vortexing to obtain homogeneous suspension. Incubate for 10 minutes at 15-25 °C.

Note: elution buffer EB is RNase-free DEPC-treated water.

2. Place the tube in magnetic rack. Incubate for 5 min.

Note: Make sure that the magnetic particles have collected on the tube wall. If a significant fraction of particles remains in the solution, increase the incubation time.

3. Transfer the supernatant into a new tube. Don't disturb magnetic particles at the tube wall.
4. It is recommended to store the eluate containing RNA at +4 °C and use it during the day for analysis. If RNA analysis is planned for another day, RNA solution should be stored at -20 °C.

Note: The analysis of isolated RNA can be performed by real-time RT-PCR. Freeze/Thaw cycles lead to RNA degradation and decreasing PCR yield.

Note: The concentration of isolated RNA cannot be determined using UV spectrometry; fluorimetric methods for determining RNA concentration are recommended.

RNA isolation protocol. Automatic protocol. Auto-Pure96 (Allsheng)

1. The plate at position #2. Lysis. Add 700 μl of the previously prepared lysing and binding mixture containing lysis buffer LB, ethanol (96-100%) and magnetic particles M to the well of the plate (**see "Before starting the procedures"**). Then add 100 μl aliquot of saline or transport medium after incubation of a cotton swabs from nose or mouth.
2. The plate at position #3. Washing. Add 500 μl of the washing buffer WB to the well of the plate.

Note: Ensure that ethanol was added to the WB.

3. The plate at position #8. RNA elution. Add 100 μl of the elution buffer EB to the well of the plate.

Note: elution buffer EB is RNase-free DEPC-treated water.

4. Place the tip comb for deep-well magnets in the plate at position #2 containing lysing and binding mixture, and the sample.
5. Run the program «BAmag_T» on the station Auto-Prep96 (Allsheng).

Note: The file "BAmag_T.txt" with the program for the Auto-Prep96 (Allsheng) station can be obtained in the following ways:

- download it yourself on the website of Biolabmix LLC (www.biolabmix.ru). Enter the product catalog number (NAmagp100) in the search bar;
- contact the sales department of Biolabmix LLC (sales@biolabmix.ru).

6. At the end of the program, the isolated RNA will be in the plate at position #8.
7. It is recommended to store the eluate containing RNA at +4 °C and use it during the day for analysis. If RNA analysis is planned for another day, RNA solution should be stored at -20 °C.

Note: The analysis of isolated RNA can be performed by real-time RT-PCR. Freeze/Thaw cycles lead to RNA degradation and decreasing PCR yield.

Note: The concentration of isolated RNA cannot be determined using UV spectrometry; fluorimetric methods for determining RNA concentration are recommended.

RNA isolation protocol. Automatic protocol. KingFisherFlex (ThermoScientific).

1. The plate #1. Lysis. Add 700 μl of the previously prepared lysing and binding mixture containing lysis buffer LB, ethanol (96-100%) and magnetic particles M to the well of the plate (**see "Before starting the procedures"**). Then add 100 μl aliquot of saline or transport medium after incubation of a cotton swabs from nose or mouth.
2. The plate #2. Washing. Add 500 μl of the washing buffer WB to the well of the plate.

Note: Ensure that ethanol was added to the WB.

3. The plate #3. RNA elution. Add 100 μl of the elution buffer EB to the well of the plate.

Note: elution buffer EB is RNase-free DEPC-treated water.

4. Place the comb in the plate #1 containing lysis buffer and the sample.
5. Run the program « BKmag_T_EtOH» on the station KingFisherFlex (ThermoScientific).

Note: The file "BKmag_T_EtOH.bdz" with the program for the KingFisher Flex station (ThermoScientific) can be obtained in the following ways:

- download it yourself on the website of Biolabmix LLC (www.biolabmix.ru). Enter the product catalog number (NAmagp100) in the search bar;
- contact the sales department of Biolabmix LLC (sales@biolabmix.ru).

6. At the end of the program, the isolated RNA will be in the plate #3.
7. It is recommended to store the eluate containing RNA at +4 °C and use it during the day for analysis. If RNA analysis is planned for another day, RNA solution should be stored at -20 °C.

Note: The analysis of isolated RNA can be performed by real-time RT-PCR. Freeze/Thaw cycles lead to RNA degradation and decreasing PCR yield.

Note: The concentration of isolated RNA cannot be determined using UV spectrometry; fluorimetric methods for determining RNA concentration are recommended.

Storage

The kit can be stored at room temperature (15-25 °C). Magnetic particles M should be stored at 2 °C to 8 °C. See expiration date on the package label.

Shipping

All components of the kit are shipped at room temperature (15-25 °C). Allowed shipping for 14 days at a temperature below 25 °C.