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Column total RNA isolation kit (RUplus)

Cat. No. RUplus-10, RUplus-50, RUplus-250

Important!

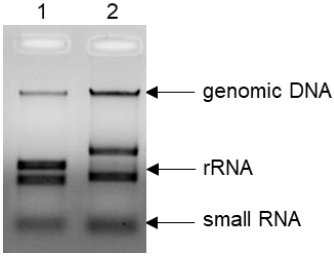
We are regularly improving the protocol for working with the kit. Please use the protocol included with the kit. The latest version of the protocol is available on the website of Biolabmix LLC (www.biolabmix.ru).
The kit is intended for research purposes only.
The protocol was updated on February 2024.

Description

The kit is designed for total RNA isolation and purification from following samples:

1. Animal cell cultures;
2. Gram-negative and gram-positive bacterial cell cultures;
3. Swabs and scrapes of epithelial cells;
4. Viruses.

The method of RNA isolation is based on the selective binding of nucleic acids from a lysed sample on a silica-gel membrane, followed by washing and elution of the purified RNA. The integrity of RNA is preserved in the process of isolation. Column capacity is up to 100 µg of RNA per one column. RNA yield depends on a sample type.
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.



Lane 1 – RNA isolated from bacterial cells, 1 mg of *E. coli.*

Lane 2 – RNA isolated from human cells, $1*10^6$ cells.

Contents

	RUplus-10 10 preps	RUplus-50 50 preps	RUplus-250 250 preps	
			Var. 1	Var. 2
Lysis buffer LB	5.5 ml	30 ml	3x50 ml	2x75 ml
Binding buffer BB	5.5 ml	30 ml	3x50 ml	2x75 ml
Wash buffer WB1	5.5 ml	30 ml	3x50 ml	2x75 ml
Wash buffer WB2 (concentrate)	1.1 ml	6 ml	3x10 ml	2x15 ml
Elution buffer EB	5 ml	15 ml	60 ml	60 ml
Lysozyme dissolving buffer	400 µl	2 ml	10 ml	10 ml
Collection tubes and spin columns	10 pcs	50 pcs	250 pcs	250 pcs

The RUplus-250 kit is supplied in one of two package variants.

Safety information

Caution! Lysis LB and wash WB1 buffers contain chaotropic salt solution, which is irritating and toxic if it comes in contact with skin or inside, causing burns. When working, always wear a suitable lab coat, disposable gloves, and protective goggles.
Caution! Binding BB and wash WB1 buffers contain isopropanol, which is irritating and toxic. Do not work with these solutions near open flames.
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, BB, WB1, WB2, EB, lysozyme dissolving buffer 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.

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

- Microcentrifuge for 1.5-2 ml tubes, speed 10000 rcf;
- Vortex;
- Single-channel variable volume micropipettes with disposable tips;
- Disposable gloves;
- 1.5 ml microcentrifuge tubes;
- Ethanol, 96-100%;
- Optional:
  14.3 M 2-mercaptoethanol (2-ME) (commercially available solutions typically contain 14.3M) or 2 M dithiothreitol (DTT) water solution as alternative;
- Optional:
  Lysozyme for RNA isolation from gram-positive bacteria;
- Optional:
  polyA RNA, for RNA isolation from a small amount of the sample (with RNA yields 1.0-1.5 μg and less).

Before starting the procedures

Prepare lysis buffer LB

- Option 1.
  Add 10 μl of 2-mercaptoethanol (2-ME) to 1 ml of LB. LB with 2-ME can be stored at 15-25°C for up to 1 month.
- Option 2.
  Add 20 μl of dithiothreitol (DTT) water solution to 1 ml of LB. LB with DTT can be stored at 15-25°C for up to 1 month. Use either freshly prepared 2 M DTT solution or frozen for single-use aliquots.

Prepare wash buffer WB2

- 1 prep, 500 μl WB2. Add 400 μl of ethanol (96-100%) to 100 μl of WB2 (concentrate).
- 10 preps. Add 4.4 ml of ethanol (96-100%) to 1.1 ml of WB2 (concentrate).
- 50 preps. Add 24 ml of ethanol (96-100%) to 6 ml of WB2 (concentrate).
- 250 preps. Var. 1. Add 40 ml of ethanol (96-100%) to 10 ml of WB2 (concentrate).
- 250 preps. Var. 2. Add 60 ml of ethanol (96-100%) to 15 ml of WB2 (concentrate).

It is recommended to add ethanol to the aliquots of the WB2, since ethanol may partially evaporate when storing the buffer for several months.

Prepare a 50 mg/ml lysozyme solution (optional)

When isolating RNA from gram-positive bacteria, prepare a lysozyme solution with a concentration of 50 mg/ml using a lysozyme dissolving buffer (included in the kit): 50 мМ Tris-HCl (pH 8), 10 мМ EDTA (pH 8), 50% glycerol. Lysozyme is not included.

To dissolve lysozyme in buffer, vortex the mixture thoroughly and incubate at room temperature for 30 min on a shaker or stirring occasionally till completely dissolved. Store the solution of lysozyme at -20°C 6 months.

Prepare a 5 mg/ml polyA RNA solution (optional)

If expected RNA yields after isolation is less than 1-2 μg (for example, 1 μg of RNA is isolated from $1*10^5$ of cultural human cells) it is recommended to prepare a 5 mg/ml water solution of polyA RNA (not included).
Store the solution of polyA at -20 °C or less. Aliquot before freezing. Each aliquot can withstand not more than 2-4 defrosting cycles.

RNA isolation protocol

1) Preparing and lysing the samples

Animal or bacterial cells

1. Resuspend the pellet of cell culture in 50 µl PBS.

Note: do not use more than $3*10^6$ animal cells or lymphocytes and not more than $1*10^8$ bacterial cells.

Note: when isolating RNA from gram-positive bacteria add 30 µl of lysozyme solution (50 mg/ml in lysozyme dissolving buffer). Incubate for 10 min at room temperature (15-25°C).

2. Add 350 µl LB.

Optional: when isolating RNA from a small amount of sample ($1*10^5$ or less of cultural animal cells or equal amount of other samples) add 5 µl (5 mg/ml) polyA to increase RNA yields.

3. Mix thoroughly by pipetting, avoid foaming.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at room temperature (15-25°C).

Swabs and scrapes of epithelial cells

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 300 µl LB.
3. Mix thoroughly by pipetting, avoid foaming.
4. Discard droplets by short centrifugation.
5. Incubate for 10 min at room temperature (15-25°C).

2) Column loading

1. Centrifuge the lysate for 10 min, 10000 rcf. Gently transfer the supernatant into new 1.5 – 2 ml tube (not included).
2. Add 400 µl BB. Mix by pipetting or short vortexing before loading to the column.
3. Transfer not more than 800 µl of the sample to the column.
4. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.

Note: if there is residual solution in a column after centrifugation, repeat the centrifugation step by increasing the speed or time of centrifugation before loading next portion of the sample.

Note: If the sample volume is more than 800 µl, transfer the excess on the same column and repeat centrifugation.

3) Column wash

1. Add 500 µl WB1 to the column. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.
2. Add 500 µl WB2 to the column. Centrifuge for 30 s, 10000 rcf. Discard the flow-through.

Note: ensure that ethanol was added to the WB2.
3. Centrifuge column for 3 min, 10000 rcf to completely remove the WB2.

4) RNA elution

1. Transfer the column into a new 1.5 ml microcentrifuge tube (not included).
2. Carefully apply 60-200 µl EB directly to the center of the column membrane. Incubate for 1 min at room temperature (15-25 °C). Centrifuge for 1 min, 10000 rcf.
   - Increasing the elution volume leads to higher RNA yields and lower RNA concentration.
   - Repeating the elution step with new aliquot of EB or reloading the eluted sample to the column allows to increase RNA yields.
   - Elution buffer EB is RNase-free DEPC-treated water.
3. Store the eluate containing RNA at -20 or -80 °C.

Optional. Treat the isolated RNA with DNase if it is necessary.

RNA analysis

RNA can be analyzed by gel electrophoresis in 1% agarose gel.
The amount of isolated RNA can be estimated using UV spectrometry.
The maximum of absorption for RNA corresponds to λ = 260 nm.
RNA concentration (µg/ml) can be calculated using the following formula:
A_{260} * dilution * 40 µg/ml.
Typical optical density ratios are A_{260}/A_{280} ~ 1.8 – 2.0.

Additional ordering Information

- Buffers for agarose gel electrophoresis: TAE (Cat. No. BE-DNA-500, BE-DNA-1000), TBE (Cat. No. TBE-500).
- Ethidium bromide (Cat. No. EtBr-10) for nucleic acid visualisation gel electrophoresis.
- Nucleic acids loading buffers (Cat. No. D-3001, D-3002, D-3003) for agarose gel electrophoresis.

Storage

All components of the kit can be stored at room temperature (15-25 °C). See expiration date on the package label.

Shipping

All components of the kit are shipped at room temperature (15-25 °C).