ExtractNow™ RNA Mini Kit

Purification of total RNA from eukaryotic and microbial materials

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The ExtractNow™ RNA Mini kit is a universal tool for rapid and efficient isolation of total RNA from various sources. Up to 20 mg of tissue, or up to $5x10^6$ eukaryotic cells, or up to $1x10^9$ bacteria can be used to obtain up to 100 µg of RNA. The kits comes with a dual spin column system to remove genomic DNA which eliminates the need of DNAse treatment. The purified RNA is free of contaminants and suitable for many downstream applications.

PRINCIPLE OF THE METHOD

The method is simple and consists of five general steps: (1) tissue homogenisation and cell lysis, (2) selective removal of genomic DNA by using spin columns (blue), (3) selective binding of RNA acids to spin column (violet), (4) removal of residual contaminants and inhibitors, and (5) elution of purified RNA. The procedure does not require phenol/chloroform extraction and needs minimal handling time. The extraction time is 15 to 40 minutes depending on the starting material.

CONTENT

Each kit contains reagents for 10 or 50 extractions. The expiry date of the unopened package is marked on the package label. Store the kit components at room temperature (18 to 25 °C). Before every use, ensure that all components have room temperature. Dissolve any precipitates in the solutions by moderate warming.

Kit component	10 Extractions (603-1010)	50 Extractions (603-1050)
Spin columns (blue)	10 units	50 units
Spin columns (violet)	10 units	50 units
Collection tubes	50 units	5 x 50 units
Lysis Buffer D	15 ml	30 ml
Binding Buffer C	16 ml	16 ml
Wash Buffer C	5 ml (add 5 ml ethanol (>96%) before first use)	15 ml (add 15 ml ethanol (>96%) before first use)
Wash Buffer D	6 ml (add 24 ml ethanol (>96%)	16 ml (add 64 ml ethanol (>96%)
RNAse-free water	2 ml	3 x 2 ml

The LOT-specific QC certificate (*Certificate of Analysis*) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The ExtractNow™ RNA Mini kit contains reagents for isolating total RNA from various sources. Additional consumables and equipment is supplied by the user:

- Ethanol (70% and > 96 % abs.)
- Isopropanol (optional)
- Bidest water
- 1.5 ml tubes
- 2 ml tubes (optional)
- Microcentrifuge and heat block or thermomixer for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (100 and 1000 μl)
- Lysozym (optional)
- TE Buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) (optional)

SPECIMEN

Best results are obtained with fresh or fresh frozen material. Repeated freeze/thaw-cycles must be avoided as it is detrimental to RNA integrity.

It is also important not to overload spin columns. The maximum amounts of starting material are:

- up to 20 mg of tissue/biopsy samples
- up to 5 x 10^6 eukaryotic cells
- 1 x 10^9 bacteria, gram(+) and gram(-)

Generally, RNA is less stable than DNA and particularly sensitive to degradation by RNAses. It is therefore essential to follow these recommendations:

- Always wear disposable laboratory gloves while handling the samples and reagents. Change gloves frequently.
- Keep samples as well as isolated RNA on ice.
- Use only RNAse-free tubes and RNAse-free filter tips.
- Do not handle any kind of cell cultures in the same laboratory where the RNA isolation will be conducted.
- Clean bench and pipettes with a RNAse decontamination solution (e.g. DNA Remover).
- All buffers and solutions should be prepared with RNAse-free water.

PRECAUTIONS

The ExtractNow™ RNA Mini kit is for research use only. The kit should be used by trained laboratory staff only.

All samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat, disposable gloves, and protective goggles.

In case of contact, flush eyes or skin with water. Do not swallow components of the kit. Clean with suitable laboratory detergent and water, if any liquid is spilt.

This kit can be disposed of as municipal waste according to local guidelines.

IMPORTANT NOTES

- Use isopropanol instead of 70% ethanol for isolating small RNAs (e.g. microRNA) as well.
- Ensure that ethanol was added to Wash Buffer C and Wash Buffer D. Do not use other alcohol apart from ethanol as it will lead to inconsistent yields. Keep the Wash Buffer vials always firmly closed.
- The centrifugation steps should be carried out at room temperature.

The reagents supplied should not be mixed with reagents from different LOT but used as an integral unit. The reagents of the kit must not be used beyond shelf life.

Follow the exact protocol. Any deviation may affect the results.

PROCEDURE

Protocol 1: RNA extraction from tissue /biopsy samples

⇒ Before first use reconstitute Wash Buffer C and Wash Buffer D with absolute ethanol.

⇒ Use a standard homogenizer (e.g. rotor-stator homogenizer, bead mill, etc.) or disrupt the tissue with mortal and pestle.

1.1	Homogenisation using a rotor-stator homogenizer: Transfer the weighed amount of tissue sample in a suitable reaction tube. Add 450 µl Lysis Buffer D and homogenize the sample thoroughly. Note: Complete homogenisation is important to maximize the RNA yield. Alternatively: Transfer the weighed amount of tissue under liquid nitrogen in a mortar and grind the sample to a fine powder. Do not allow the sample to thaw! Transfer the powder into a 1.5 ml reaction tube and add 450 µl Lysis Buffer D. Incubate the sample continuously shaking at room temperature for further lysis. Proceed with the next step or store the sample in Lysis Buffer D at –20 °C.
1.2	After lysis centrifuge the tube at max. speed for 1 min. to pellet any unlysed material.
1.3	Pipet the supernatant to a spin column (blue) placed in a collection tube. Close the cap and centrifuge at 10,000 x g for 2 min. If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation. **Important:** The flow-through contains the RNA. Thus do not discard the flow-through!
1.4	Discard the spin column (blue) and place a spin column (violet) in a new collection tube.
1.5	Add an equal volume (~400 µl) of 70 % ethanol to the flow-through from step 1.3 and mix thoroughly by pipetting up and down. Alternatively: add isopropanol instead of 70 % ethanol to the flow-through if you want to extract small RNAs as well. Mix thoroughly.
1.6	Transfer the sample into the spin column (violet) and centrifuge at 10,000 x g for 2 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.

1.7	Discard the collection tube and place the spin column in a new collection tube.
1.8	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
1.9	Add 700 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
1.10	Centrifuge at 10,000 x g for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
1.11	Add 30 to 80 µl but at least 20 µl RNAse-free water and incubate at room temperature for 1 min.
1.12	Centrifuge at 6000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Store the extracted RNA at –20 °C or at –80 °C for long time storage.

Protocol 2: RNA extraction from eukaryotic cells

⇒ Before first use reconstitute Wash Buffer C and Wash Buffer D with absolute ethanol.
⇒ Do not use more than 5 x 10^6 cells.

2.1	Pellet your cells and discard the supernatant. Add 400 µl Lysis Buffer D to the pellet and incubate the sample for 2 min at room temperature. Resuspend the cell pellet by pipetting up and down. Incubate for further 3 min at room temperature. Note: ensure that all cells are completely lysed (no visible cell clumps) before you proceed to the next step.
2.2	Pipet the lysed sample to a spin column (blue) placed in a collection tube. Close the cap and centrifuge at 10,000 x g for 2 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation. **Important:** The flow-through contains the RNA. Thus do not discard the flow-through!
2.3	Discard the spin column (blue) and place a spin column (violet) in a new collection tube.
2.4	Add an equal volume (~400 µl) of 70 % ethanol to the flow-through from step 2.2 and mix thoroughly by pipetting up and down. Alternatively: add isopropanol instead of 70 % ethanol to the flow-through if you want to extract small RNAs as well. Mix thoroughly.
2.5	Transfer the sample into the spin column (violet) and centrifuge at 10,000 x g for 2 min. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
2.6	Discard the collection tube and place the spin column in a new collection tube.
2.7	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
2.8	Add 700 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.

2.9	Centrifuge at 10,000 x g for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
2.10	Add 30 to 80 µl but at least 20 µl RNAse-free water and incubate at room temperature for 1 min.
2.11	Centrifuge at 6000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Store the extracted RNA at –20 °C or at –80 °C for long time storage.

Protocol 3: RNA extraction from bacteria

⇒ Before first use reconstitute Wash Buffer C and Wash Buffer D with absolute ethanol.
⇒ Do not use more than 1 x 10^9 bacteria.
⇒ We recommend a pre-incubation step with lysozyme or another lysis protein.

3.1	Prepare a stock solution of lysozyme for: A) gram(-) bacteria: 20 mg/ml in water, or B) gram(+) bacteria: 50 mg/ml in water. Aliquot the stock solution and store the aliquots at –20 °C. Prepare TE Buffer.
3.2	Pellet the bacterial cells for 2 to 5 min at 5000 x g. Remove the supernatant as much as possible.
3.3	For **gram(-) bacteria**: resuspend the cell pellet in 100 µl TE Buffer and add 2 µl of the corresponding lysozym stock solution (20 mg/ml), or for **gram(+) bacteria**: resuspend the cell pellet in 100 µl TE Buffer and add 6 µl of the corresponding lysozym stock solution.
3.4	Mix by pipetting and incubate until the solution becomes clear or viscous. Note: the specific amount of lysozym as well as the incubation time may need to be adjusted in relation to the particular bacterial strain.
3.5	Add 450 µl Lysis Buffer D and vortex the sample thoroughly. Incubate the sample for 3 min at room temperature. Note: ensure that all cells are completely lysed (no visible cell clumps) before you proceed to the next step.
3.6	Pipet the lysate to a spin column (blue) placed in a collection tube. Close the cap and centrifuge at 10,000 x g for 2 min. If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation. **Important:** The flow-through contains the RNA. Thus do not discard the flow-through!
3.7	Discard the spin column (blue) and place a spin column (violet) in a new collection tube.

3.8	Add an equal volume (~600 µl) of 70 % ethanol to the flow-through from step 3.6 and mix thoroughly by pipetting up and down. Alternatively: add isopropanol instead of 70 % ethanol to the flow-through if you want to extract small RNAs as well. Mix thoroughly.
3.9	Transfer 650 µl of the sample into the spin column (violet) and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube. Load the remaining volume and centrifuge again. Discard the collection tube and place the spin column in a new collection tube. Note: If the solution has not completely passed through the spin column, centrifuge again at a higher speed or prolong the centrifugation.
3.10	Add 500 µl Wash Buffer C and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
3.11	Add 700 µl Wash Buffer D and centrifuge at 10,000 x g for 1 min. Discard the collection tube and place the spin column in a new collection tube.
3.12	Centrifuge at 10,000 x g for 3 min to remove all traces of ethanol. Discard the collection tube and place the spin column in a new 1.5 ml tube.
3.13	Add 30 to 80 µl but at least 20 µl RNAse-free water and incubate at room temperature for 1 min.
3.14	Centrifuge at 6000 x g for 1 min. A second elution step will increase the yield of extracted RNA. Store the extracted RNA at –20 °C or at –80 °C for long time storage.

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from the use, the results of use, or the inability to use this product.

Related Products

DNA Extraction kits

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions

MB *Taq* DNA Polymerase

53-0050/0100/0200/0250	MB *Taq* DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/1100/1200/1250	MB *Taq* DNA Polymerase (1 U/µl)	50/100/200/250 units

Contamination Control PCR kits

11-1025/1050/1100/1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/7048/7096/7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/8050/8100/8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/1050/1100/1250	Onar® Bacteria Detection Kit	25/50/100/250 tests
11-9025/9100/9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 tests

Mycoplasma Elimination

10-0200/0500/1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 1x10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumonia*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See Minerva homepage for further available species

DNA Remover™

15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 wipes

Mycoplasma Off

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 x 1000 ml
15-1001	Surface Disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

13-0050/0150	Contamination Prevention Reagent 100x concentrate	1000 ml/ 5 x 1000 ml

WaterShield™

15-3025/3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

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