Total RNA Purification Kit

Catalog Number: orb533444 (Product Manual)

Description:

Total RNA Purification Kit is designed for rapid, high purity and high yield isolation of total RNA from small amounts of various samples including blood, animal and plant tissue, bacteria and viruses.

The spin column based method allows complete removal of inhib-itors such as divalent cations and proteins. Due to elimination of phenol, handling of the kit is safe and no harmful waste is produced. The purified total RNA can be used in a number of downstream applications. The kit allows the purification of up to 100 µg RNA per preparation.

Content:

Lysis Buffer (before use, add 2-Mercaptoethanol as indicated on the bottle) - stable for 1 month at room temperature. Activation Buffer

Blood Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)

First Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)

Second Washing Buffer (before use, add 96-99 % Ethanol as indicated

on the bottle)

Elution Buffer

Spin Columns

2 ml Collection Tubes

To be provided by you:

2-Mercaptoethanol (2-ME)

Optional: Chloroform

96-99 % Ethanol

2-Propanol (Isopropanol)

1.5 ml microtubes

Preparation procedure:

Before start, add the following components (not included in the kit) as indicated on the respective bottle:

- 2-Mercaptoethanol to the Lysis Buffer (10 µl 2-Mercaptoethanol per 1 ml Lysis Buffer)
- 96-99 % Ethanol to Blood Washing Buffer, First Washing Buffer and Second Washing Buffer

Buffer	**PP-210XS** 10 preps	**PP-210S** 50 preps	**PP-210L** 250 preps
Lysis Buffer	5.2 ml (add 52 µl 2-ME)	26 ml (add 260 µl 2-ME)	130 ml (add 1.3 ml 2-ME)
Activation Buffer	1.2 ml	6 ml	30 ml
Blood Washing Buffer	Add 6.4 ml Ethanol (final volume 8 ml)	add 32 ml Ethanol (final volume 40 ml)	add 160 ml Ethanol (final volume 200 ml)
First Washing Buffer	add 1.6 ml Ethanol (final volume 8 ml)	add 8 ml Ethanol (final volume 40 ml)	add 40 ml Ethanol (final volume 200 ml)
Second Washing Buffer	add 6.4 ml Ethanol (final volume 8 ml)	add 32 ml Ethanol (final volume 40 ml)	add 160 ml Ethanol (final volume 200 ml)
Elution Buffer	1 ml	5 ml	25 ml

1 Sample Preparation and Cell Lysis:

Blood

- Transfer 100 µl of non-coagulating blood to a microcentrifuge tube.

- Add 500 µl of Lysis Buffer (2-ME added) and vortex for 10 sec.

Fresh Tissue Sample - Animals or Plants

- Collect 20-50 mg fresh tissue sample in a micro-centrifuge tube.
- Add 300 µl of Lysis Buffer (2-ME added) and homogenize the material using an appropriate apparatus (hand-operated pellet pestle or motor-driven grinder).
- Add additional 200 µl of Lysis Buffer (2-ME added) to the ho-mogenized sample and vortex 15-30 sec (Note: Sample volume should not exceed 10 % of the Lysis Buffer volume).
- Centrifuge at 10,000 g for 10 min.

Optional step in case that debris still remains in the supernatant:

- Add 500 µl chloroform (not included in the kit) and vortex for 15-30 sec.
- Centrifuge at 10,000 g for 10 min.
- Transfer the supernatant (if you added chloroform: the upper aqueous phase) into a microcentrifuge tube.

Cells from Nasal or Throat Swabs

- Add 500 µl of Lysis Buffer (2-ME added) to a microcentrifuge tube.
- Brush a sterile, single-use cotton swab or Buccal Swab Brush inside the nose or mouth of the subject.
- Cut the cotton tip where the nasal or throat cells were collected and place it into the microcentrifuge tube containing the Lysis Buffer (2-ME added).
- Close the tube, vortex and incubate at room temperature for 5 min.

Cells Grown in Monolayer

- Put off culture media.
- Add 500 µl of Lysis Buffer (2-ME added) per 1-5 x 106 cells.
- Lyse cells and homogenize the sample by pipetting up and down several times.

Cells Grown in Suspension

- Pellet 1-5 x 106 animal, plant or yeast cells, or 1 x 107 bacterial cells. (Occasionally, enzymatic lysis or mechanical disruption is required for cell-wall disruption of some yeast and bacterial cells.)
- Discard the supernatant and add 500 µl of Lysis Buffer (2-ME added).
- Lyse the sample by repetitive pipetting or vortexing for 10 sec.

2 Column Activation [optional]:

- Place a spin column into a 2 ml collection tube.
- Add 100 µl Activation Buffer into the Spin Column.
- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.
- Immediately proceed to next step.

3 Column Loading:

- Add 300 µl (or 0.6 x volume of the cell lysate) Isopropanol to the prepared lysate and vortex.
- Transfer the mixture directly into the spin column.
- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.

4 First Column Washing:

Preparation from blood

- Apply 700 µl of Blood Washing Buffer (ethanol added) to the Spin Column.
- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.

Preparation from tissue, swabs or cell culture

- Apply 700 µl of First Washing Buffer (ethanol added) to the Spin Column.

- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.

5 Second Column Washing:

- Apply 700 µl of Second Washing Buffer (ethanol added) to the Spin Column.
- Centrifuge at 10,000 g for 30 sec.
- Discard the flow-through.
- Centrifuge again at 10,000 g for 2 min to remove residual eth-anol.

6 Elution of RNA:

- Place the Spin Column into a DNase/RNase-free microcentri-fuge tube.
- Add 40-50 µl Elution Buffer to the center of the column mem-brane.
- Incubate at room temperature for 1 min.
- Centrifuge at 10,000 g for 1 min to elute the RNA
- Store RNA at -20 or -80 °C.

Elimination of remaining DNA:

Remaining genomic DNA may be particularly a problem in sub-sequent RT-PCR or quantification of low-copy transcripts. For complete removal of gDNA from RNA preparations Biorbyt gDNA Removal Kit (Cat.-No. PP-219) is recommended.The kit is based on a heat labile dsDNase which is irreversibly inactivated at moderate temperatures.