

Total RNA Purification Kit

Isolation of total RNA by silica-gel membrane adsorption

RNA Purification

	Cat.-No.	Amount
	PP-210XS	10 preparations
	PP-210S	50 preparations
	PP-210L	250 preparations

For *in vitro* use only

Quality guaranteed for 12 months

Store at room temperature

2-Mercaptoethanol containing *Lysis Buffer* is stable for 1 week at room temperature

Kit contents

Lysis Buffer (before use, add 2-Mercaptoethanol as indicated on the bottle)

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

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

Elution Buffer

Spin Columns and 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

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 kit is also recommended for isolation of total RNA from virus-infected tissue samples.

The spin column based method allows complete removal of inhibitors 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.

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 (Please note: 2-Mercaptoethanol containing *Lysis Buffer* is stable for 1 week at RT)
- 96-99% Ethanol to the Primary Washing Buffer
- 96-99% Ethanol to the Secondary Washing Buffer

Buffer	PP-210XS	PP-210S	PP-210L
	10 preps	50 preps	250 preps
Lysis Buffer	add 60 µl 2-ME (final vol. 6,06 ml)	add 300 µl 2-ME (final vol. 30,3 ml)	add 1,5 ml 2-ME (final vol. 151,5 ml)
Primary Washing Buffer	add 1,6 ml Ethanol (final vol. 8 ml)	add 8 ml Ethanol (final vol. 40 ml)	add 40 ml Ethanol (final vol. 200 ml)
Secondary Washing Buffer	add 6,4 ml Ethanol (final vol. 8 ml)	add 32 ml Ethanol (final vol. 40 ml)	add 160 ml Ethanol (final vol. 200 ml)
Elution Buffer	1 ml	5 ml	25 ml

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RNA Purification

1 Sample Preparation and Cell Lysis

Fresh Tissue Sample – Animals or Plants

- Collect 20-50 mg fresh tissue sample in a microcentrifuge tube
- Add 300 µl of *Lysis Buffer* (containing 2-ME) and homogenize the material using an appropriate apparatus (hand-operated pellet pestle or motordriven grinder)
- Add additional 200 µl of *Lysis Buffer* (containing 2-ME) to the homogenized sample and vortex 15-30 sec (Note: Sample volume should not exceed 10% of the *Lysis Buffer* volume)
- Centrifuge at 12,000 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 12,000 g for 10 min
 - Transfer to a microcentrifuge tube
- Transfer the supernatant (if you added chloroform: the upper aqueous phase) into a microcentrifuge tube

Blood

- Transfer 100 µl of non-coagulating blood to a microcentrifuge tube
- Add 500 µl of *Buffer Lysis Buffer* (2-ME added) and vortex at 12,000 g for 10 sec

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 $1-5 \times 10^6$ cells
- Lyse cells and homogenize the sample by pipetting up and down several times

Cells Grown in Suspension

- Pellet $1-5 \times 10^6$ animal, plant or yeast cells, or 1×10^7 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 Loading

- Add 300 µl (or 0.6 x volume of the cell lysate) Isopropanol to the prepared cell lysate and vortex
- Place a *Spin Column* into a 2 ml collection tube
- Transfer the mixture from step 2 directly into the spin column
- Centrifuge at 12,000 g for 30 sec
- Discard the flow-through

Optional: RNase-free DNase can be applied with on-column DNase Digestion for the elimination of remaining genomic DNA

3 Primary Column Washing

- Apply 700 µl of *Primary Washing Buffer* (ethanol added) to the *Spin Column*
- Centrifuge at 12,000 g for 30 sec
- Discard the flow-through

4 Secondary Column Washing

- Apply 700 µl of *Secondary Washing Buffer* (ethanol added) to the *Spin Column*
- centrifuge at 12,000 g for 30 sec
- Discard the flow-through
- Centrifuge again at 12,000 g for 2 min to remove residual ethanol

5 Elution of RNA

- Place the *Spin Column* into an RNase-free microcentrifuge tube
- Add 40-50 µl *Elution Buffer* to the center of the column membrane
- Incubate at room temperature for 1 min
- Centrifuge at 12,000 g for 1 min to elute the RNA
- Store RNA at -20 or -80°C