WizPrep™ Total RNA Mini Kit (Cell)

RUO For Research Use Only

REF W72060

DESCRIPTION

The WizPrep™ Total RNA Mini Kit (Cell) provides a fast and simple method to isolate total RNA from various cultured cells and bacteria.
The WizPrep™ Total RNA Mini Kit (Cell) uses silica-membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. The kit is ready for use and can purify the total RNA from a wide variety of animal cell and bacterial samples, and the whole process is completed in less than 20 minutes.
Purified RNA is suitable for RT-PCR, northern blotting, primer extension and cDNA library construction.

KIT CONTENTS

Contents	100 prep	300 prep	Storage
RC Buffer	25 ml	80 ml	Room temp.
RL Buffer	35 ml	110 ml	Room temp.
W1 Buffer	60 ml	180 ml	Room temp.
W2 Buffer (concentrate)(1)	30 ml	90 ml	Room temp.
RNase-Free Water	10 ml	30 ml	Room temp.
Spin Columns*	100	300	Room temp.
Instruction Manual	1	1	

(1) : Add absolute ethanol to the W2 Buffer prior to initial use (see the bottle label for volume).
* All Spin Columns are sterilized by electron beam.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY USER

- 96-100% ethanol (to prepare W2 Buffer)
- 1.5 ml microcentrifuge tubes
- Sterile RNase-free pipette tips and Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)
- Lysozyme Solution : 20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0 (Prepare the lysozyme mix immediately prior to use)

KIT SPECIFICATIONS

Parameter	Characteristics
Format	Silca-membrane spin column
Sample materials	Cultured cells (< 10^6) or Bacterial cell (< 10^9)
Typical yield	5 - 35 µg (depending on sample)
Elution volume	50 µl
Preparation time	< 20 minutes

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPrep™ Total RNA Mini Kit (Cell) is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

To check before start

1) Add absolute ethanol to the W2 Buffer prior to initial use (see the bottle label for volume)
2) If a precipitate has formed in RL Buffer, dissolve by incubating at 56℃ before use.

A. Cultured cells and Gram-negative Bacteria

- Transfer the cultured cells (up to 1 x 10^6) or bacterial culture (up to 1 x 10^9) to a 1.5 ml tube.
- Centrifuge at 13,000 rpm for 1 min to pellet the cells and carefully remove the supernatant.
- Add **200 µl of RC buffer** to the pellet and completely resuspend the pellet by vortexing or pipetting. Store at R/T for 5 min.
- Add **300 µl of RL Buffer** and **5 µl of β-mercaptoethanol** and vortex vigorously. Store at R/T for 5 min.
- Centrifuge at 13,000 rpm for 2 min.
- Transfer the supernatant (400 µl) to new 1.5 ml tube.

▶ *Proceed to Step 2.*

B. Gram-positive Bacteria

- Transfer the bacterial culture (up to 1 x 10^9) to a 1.5 ml tube.
- Centrifuge at 13,000 rpm for 1 min and remove the supernatant completely.
- Add **200 µl of Lysozyme Solution** (20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0) to the tube and resuspend the cell pellet by vortex or pipetting.
- Incubate at R/T for 10 min. During incubation, invert the tube every 2-3 minutes.
- Add **300 µl of RL Buffer, 5 µl of β-mercaptoethanol** and homogenize the sample tissue by grinding. Store at R/T for 5 min.
- Centrifuge at 13,000 rpm for 1 min.
- Transfer the supernatant (400 µl) to new 1.5 ml tube.

▶ *Proceed to Step 2.*

Step 2 : Binding

- Add **400 µl of 70% Ethanol** to filterate and mix by pipetting 5 times.
- Connect Spin Column to Collection tube.
- Apply the mixture to the Spin Column and centrifuge at 13,000 rpm for 1 min.
- Discard the flow-through and re-connect with the Spin Column.

(Optinal) DNA residue degradation

Add 100µl of DNase I solution (2U/µl) in center of Spin Column matrix and incubate at R/T for 10 min.

Step 3 : Wash

- Add **500 µl of W1 Buffer** to the Spin Column and centrifuge at 13,000 rpm for 1min then discard the flow-throw
- Add **600 µl of W2 Buffer** (ethanol added) in the Spin Column and centrifuge at 13,000 rpm for 1 min then discard the flow-throw.
- Add **600 µl of W2 Buffer** (ethanol added) in the Spin Column and centrifuge at 13,000 rpm for 1 min then discard the flow-throw.
- Centrifuge at 13,000 rpm for 3 min.

Step 4 : Elution

- Connect the Spin Column and new 1.5 ml tube.
- Add **50 µl of RNase-Free Water** into the center of Spin Column and incubate at R/T for 1 min.
- Centrifuge at 13,000 rpm for 3 min.
- Discard the Spin Column
- Eluted RNA are stored at −20 °C for a few days, −70 °C for long term storage.

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TROUBLESHOOTING

Problem	Solution and Explanation
The Spin column is clogged	• Inefficient disruption and/or homogenization • Too much starting material • Centrifugation temperature was too low (should be 20-25ºC)
Low yield of RNA	• Insufficient disruption and homogenization • Too much starting material • RNA still bound to RNA spin column membrane • Ethanol carryover
RNA Degradation	• Harvested animal tissue not immediately stabilized • Inappropriate handling of starting material • RNase contamination

SYMBOL GLOSSARY

REF	Catalogue number		Manufacturer
LOT	Batch code	RUO	Research use only
	Temperature limit		Instructions for use
	Use-by date		

ORDERING INFORMATION

Product	Cat No.	Package
WizPrep™ Total RNA Mini Kit (Cell)	W72060-100	100 prep
	W72060-300	300 Prep

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