EpiQuik™ Total RNA Isolation Kit

Base Catalog # P-9100

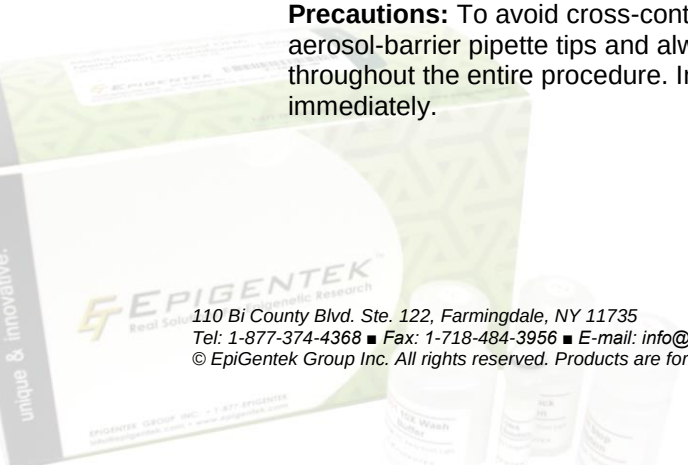
PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Total RNA Isolation Kit is suitable for a quick preparation of total RNA from blood, cultured cells, or fungus.

Input Amount: The amount of starting materials can be up to 300 µl of whole blood, $10^{\wedge 7}$ mammalian cells, $10^{\wedge 9}$ bacterial cells, and $10^{\wedge 8}$ fungal cells. A total of 50 standard extractions can be performed with this kit.

Yield: Yield of the total RNA can be up to 30 µg. The yield may vary depending on the sample type.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution using aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.



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KIT CONTENTS

Components	50 samples P-9100-050	Shipping Temperature	Storage Upon Receipt	Storage Checklist
RL (Pre-lysis Buffer)	2 x 25 ml (50 ml)	RT	RT	
RA (Lysis Buffer)	20 ml	RT	RT	
RO (Pre-lysis Buffer-Bacteria/Fungus)	10 ml	RT	RT	
W1 (Wash Buffer-1)	20 ml	RT	RT	
W2 (Wash Buffer-2) *	2 x 3 ml (add 12 ml ethanol to each bottle)	RT	RT	
RE (Elution Buffer)	5 ml	RT	RT	
R-Column	50 pcs	RT	RT	
Collection Tubes	50 pcs	RT	RT	
User Guide	1	RT	RT	

Add 12 ml ethanol (96~100%) to the each 3 ml bottle of **W2 (Wash Buffer-2), and shake before use.*

SHIPPING & STORAGE

The kit is shipped at ambient room temperature. Upon receipt: Store all components at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

***Note:** Check all buffers for salt precipitation prior to use. Re-dissolve any precipitate by warming up to 37°C.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and RNase-free pipet tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Ethanol (96-100%)
- ☐ 70% Ethanol prepared with ddH_2O (double distilled water or RNase- and DNase-free water)
- ☐ 14.3 M ß-mercaptoethanol
- ☐ *For the Optional Step (DNA Residue Degradation):* Add 2 µl of the DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5) and 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the final elution sample. Let it stand for 10 minutes at room temperature.

- ☐ *For the Gram-positive bacteria sample:* lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use).
- ☐ *For the fungus sample:* lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35 mM ß-mercaptoethanol).

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Total RNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality. EpiGentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpiGentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The EpiQuik™ Total RNA Isolation Kit is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

The *EpiQuik™ Total RNA Isolation Kit* provides a fast, simple, and cost-effective method for the isolation of total RNA from whole blood, mammalian cells, bacterial cells, and fungal cells. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species bases to bind to the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and the pure RNA is eluted without phenol extraction or alcohol precipitation. The RNA purified with the EpiQuik™ Total RNA Isolation Kit is suitable for a variety of routine applications, including RT-PCR, cDNA synthesis, northern blotting, differential display, primer extension, and mRNA selection. The entire procedure can be completed within 25-40 minutes.

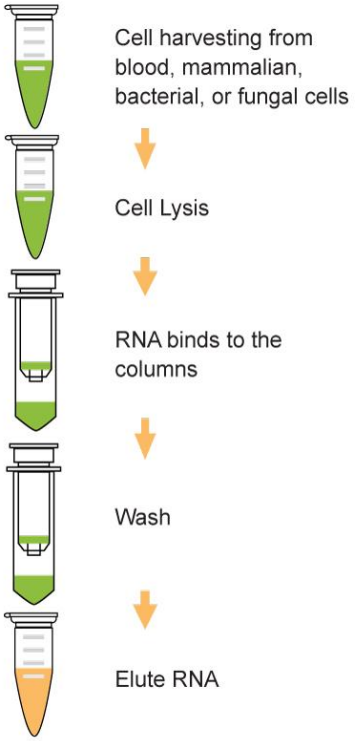
The EpiQuik™ Total RNA Isolation Kit has the following features:

- Fast procedure delivers high-quality total RNA in 25-40 minutes
- Ready-to-use RNA for high performance in any downstream application
- Consistent RNA yield from a small amount of starting material

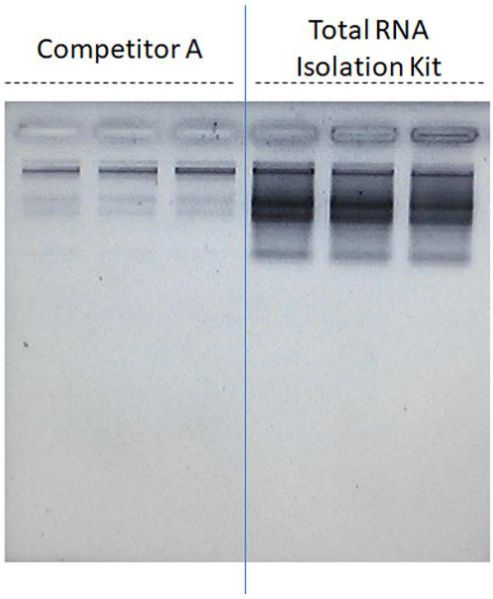
PRINCIPLE & PROCEDURE

The *EpiQuik™ Total RNA Isolation Kit* contains all the reagents required for successfully performing RNA isolation directly from blood, cultured cells, or fungus. After lysis, binding, and

wash, RNA is easily recovered in quantities of up to 30 µg using specially designed columns. Total RNA is then ready to be used for a variety of downstream applications.



▲ Schematic Procedure for the EpiQuik™ Total RNA Isolation Kit.



▲ Total RNA was both isolated from 300 µl bacteria culture.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The sample amount can be up to 300 µl of whole blood, $10^{\wedge 7}$mammalian cells, $10^{\wedge 9}$ bacterial cells, and $10^{\wedge 8}$ fungal cells.

1. Sample Cells Harvesting

Fresh Blood

a. Collect blood in EDTA-Na_2-treated collection tubes (or other anticoagulant mixtures).
b. Transfer up to 300 µl of the blood to a sterile 1.5 ml microcentrifuge tube.
c. Add 900 µl of the **RL** (Pre-lysis Buffer) and mix by inversion.
d. Incubate the tube on ice for 10 minutes (invert twice during incubation).
e. Centrifuge for 5 minutes at 4ºC.

f. Remove the supernatant completely and resuspend the cells in 100 µl of **RL** (Pre-lysis Buffer) by pipetting the pellet.

Cultured Mammalian Cells

a. Transfer the cultured mammalian cells (up to 10^7) to a sterile 1.5 ml microcentrifuge tube.
b. Centrifuge at 6,000 x g for 1 minute. Remove the supernatant completely and resuspend the cells in 100 µl of the **RL** (Pre-lysis Buffer) by pipetting the pellet.

Gram-Negative Bacterial Cells

a. Transfer the cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
b. Centrifuge at 12,000 x g for 1 minute.
c. Remove the supernatant completely and resuspend the cells in 200 µl of the **RO** (Pre-lysis Buffer) by pipetting the pellet. Incubate at room temperature for 5 minutes.

Gram-Positive Bacterial Cells

a. Transfer the cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
b. Centrifuge at 12,000 x g for 1 minute.
c. Remove the supernatant completely and resuspend the cells in 200 µl of the lysozyme buffer by pipetting the pellet. Incubate at room temperature for 10 minutes.

Fungal Cells

a. Transfer the fungal cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
b. Centrifuge at 6,000 x g for 5 minutes.
c. Remove the supernatant completely and resuspend the cells in 600 µl of the sorbitol buffer by pipetting the pellet.
d. Add 200 U of the lyticase. Incubate at 30ºC for 30 minutes.
e. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast.
f. Remove the supernatant completely and resuspend the cells in 200 µl of the **RO** (Pre-lysis Buffer) by pipetting the pellet. Incubate at room temperature for 5 minutes.

2. Lysis

Fresh Blood/Mammalian Cells

a. Add 400 µl of the **RA** (Lysis Buffer) and 4 µl of the ß-mercaptoethanol to the resuspended cells from Step 1 and shake vigorously. Incubate at room temperature for 5 minutes.
b. Centrifuge at 16,000 x g for 10 minutes.
c. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Bacterial Cells/ Fungal Cells

a. Add 300 µl of the **RA** (Lysis Buffer) and 3 µl of the ß-mercaptoethanol to the resuspended cells from Step 1 and mix by vortexing. Incubate at room temperature for 5 minutes.
b. Centrifuge at 16,000 x g for 10 minutes.
c. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

3. Binding

a. Add 500 µl of the 70% ethanol prepared with the ddH_2O (or RNase-free and DNase-free water) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting).
b. Place an **R-Column** in a **Collection Tube**. Apply 600 µl of the mixture to the **R-Column**.

c. Centrifuge at 14,000 x g for 1 minute. Discard the flow-through and place the **R-Column** in the same **Collection Tube**.
d. Transfer the remaining mixture to the same **R-Column**.
e. Centrifuge at 14,000 x g for 1 minute. Discard the flow-through and place the **R-Column** in a new **Collection Tube.**

4. Wash

a. Add 400 µl of the **W1** (Wash Buffer-1) into the **R-Column**. Centrifuge at 14,000 x g for 30 seconds.
b. Discard the flow-through and place the **R-Column** back into the same **Collection Tube**.
c. Add 600 µl of the **W2** (Wash Buffer-2) (ethanol added) into the **R-Column**.

   Note: *As shown in the Kit Contents table, remember to add 12 ml of ethanol (96~100%) to each 3 ml bottle of* ***W2*** *(Wash Buffer-2) and shake before use.*

d. Centrifuge at 14,000 x g for 30 seconds.
e. Discard the flow-through and place the **R-Column** back into the same **Collection Tube**.
f. Centrifuge at 14,000 x g again for 2 minutes to remove the residual **W2** (Wash Buffer-2).

5. Elution

a. To elute RNA, place the **R-Column** in a clean 1.5 ml microcentrifuge tube.
b. Add 50 - 100 µl of the **RE** (Elution Buffer) to the center of each **R-Column**, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

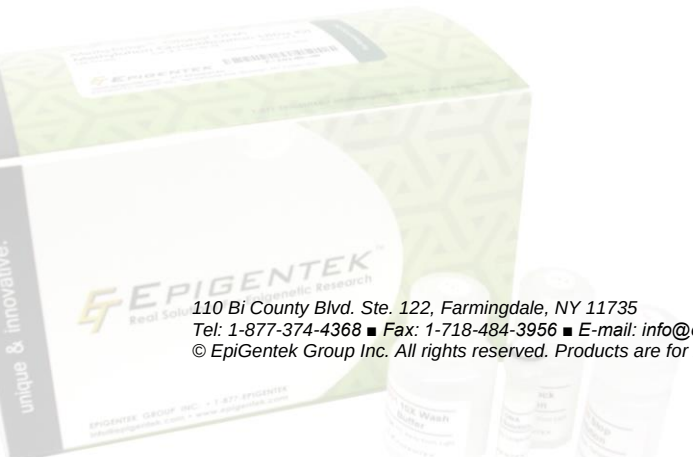
Optional Step: *DNase treatments can be followed to remove the unwanted DNA residue.*

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Degraded RNA/low integrity	RNase contaminant	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, Eg. RNase inhibitor.
Low yields of RNA	Incomplete lysis and homogenization	Use the appropriate method for the lysate preparation based on the amount of the starting materials immersed in the **RA** (Lysis Buffer) to achieve the optimal lysis.
	Incorrect elution conditions	Add 50 µl of the **RE** (Elution Buffer) to the center of each **R-Column**, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
Inhibition of downstream Enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 14,000 x g again for 2 minutes to remove the residual **W2** (Wash Buffer-2).

RELATED PRODUCTS

P-9003	Methylamp™ RNA Bisulfite Conversion Kit
P-9005	EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric)
P-9007	EpiNext™ 5-mC RNA Bisulfite-Seq Easy Kit (Illumina)
P-9008	EpiQuik™ m6A RNA Methylation Quantification Kit (Fluorometric)
P-9009	MethylFlash™ 5-mC RNA Methylation ELISA Easy Kit (Fluorometric)



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