EpiQuik™ Total RNA Isolation Fast Kit

Base Catalog # P-9105

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ Total RNA Isolation Fast Kit is suitable for quick preparation of total RNA from cultured cells or tissues.

Input Amount: The amount of starting materials can be up to 1 X 10^6 mammalian cells and 10 mg tissues. A total of 50 standard extractions can be performed with this kit.

Yield: Yield of the total RNA can be up to 10 µg from the cultured cells and tissues. 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-9105-050	Shipping Temperature	Storage Upon Receipt	Storage Checklist
RLB (RNA Lysis Buffer)	12 ml	RT	RT	
RPB (RNA Purification Buffer)	4 ml	RT	RT	
RWB (RNA Wash Buffer)	8 ml	RT	RT	
DNase I (Lyophilized)*	1 vial	RT	-20°C	
DNase Reaction Buffer	1 ml	RT	RT	
EB (Elution Buffer)	2 ml	RT	RT	
F-Spin Column	50 pcs	RT	RT	
F-Collection Tubes	50 pcs	RT	RT	

** Prior to use, reconstitute the lyophilized DNase I with 1 ml of distilled water and store at -20°C in aliquots.*

SHIPPING & STORAGE

The kit is shipped at ambient room temperature. Upon receipt: Store **DNase I** (Lyophilized) at -20°C and all other 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 (RNase-free)
- ☐ Isopropanol (100%)
- ☐ Ethanol (100%)
- ☐ Distilled water

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GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ Total RNA Isolation Fast 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. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

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

A BRIEF OVERVIEW

The *EpiQuik™ Total RNA Isolation Fast Kit* provides a fast, simple, and cost-effective method for isolating total RNA from mammalian cells and tissues. Detergents are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows protein/DNA to be removed and RNA to bind to the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and pure RNA is eluted. The RNA purified with the EpiQuik™ Total RNA Isolation Fast 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 30 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 Fast Kit* contains all the reagents required for successfully performing RNA isolation directly from cultured cells or tissues. After lysis, binding, and wash, RNA is easily recovered in quantities of up to 10 µg using specially designed columns. Total RNA is then ready to be used for a variety of downstream applications.

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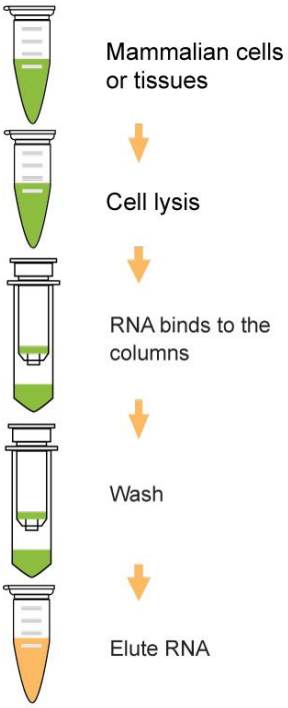

Fig 1. Schematic Procedure for the EpiQuik™ Total RNA Isolation Fast Kit.

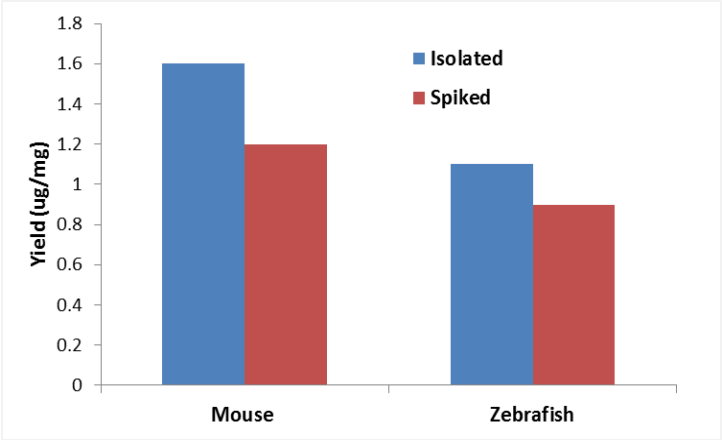

Fig 2. Total RNA was extracted from mouse liver tissue (~6 mg) and Zebrafish tissue (10 mg) using the EpiQuik™ Total RNA Isolation Fast Kit. The concentrations were quantified using a fluorescent method for RNA/ssDNA measurement. The isolated RNA was spiked into FBS and re-isolated/purified with the kit to determine the recovery efficiency. The recovered RNA was then normalized to yield unit (µg/mg).

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 10^6 mammalian cells or 10 mg tissues.

Working Buffer Preparation

Working RWB (RNA Wash Buffer): Add 32 ml of *100% Ethanol* in to 8 ml of **RWB** (RNA Wash Buffer).

DNase I working solution: For each sample reaction, mix 4 µl of reconstituted **DNase I** (Lyophilized) and 16 µl of **DRB** (DNase Reaction Buffer). The total volume should be prepared based on the needed sample quantities.

Working RPB (RNA Purification Buffer): Add 16 ml of *100% Ethanol* in to 4 ml of **RPB** (RNA Purification Buffer).

1. Sample Harvesting

Adhesive cultures: Cells (no more than 1×10^6) are detached by trypsinization and collected into a 1.5 ml vial. Centrifuge the cells at 5000 rpm for 1 min. Remove the supernatant completely and resuspend the cells in 250 µl

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of the **RLB** (RNA Lysis Buffer) by pipetting up and down. Incubate at room temperature for 5 min, followed by incubating at 4°C for an additional 5 min.

*Suspension cells***:** Cells (no more than 1×10^6) are directly collected into a 1.5 ml vial. Centrifuge the cells at 5000 rpm for 1 min. Remove the supernatant completely and resuspend the cells in 250 µl of the **RLB** (RNA Lysis Buffer) by pipetting up and down. Incubate at room temperature for 5 min, followed by incubating at 4°C for an additional 5 min.

Tissues: Harvest 10 mg or less of various tissues into a 1.5 ml tube. Add nitrogen liquid to immerse the tissue. While the nitrogen liquid is still evaporating, very gently crush the tissue clumps into small pieces with a pestle that is suitable for a 1.5 ml tube. When the nitrogen liquid has nearly evaporated, vigorously grind the tissue species into fine powder. Add 250 µl of the **RLB** (RNA Lysis Buffer) to the ground tissue and homogenize quickly by vortexing for 5 sec and inverting and flicking the tube gently. Incubate at room temperature for 5 min, followed by incubating at 4°C for an additional 5 min.

Alternatively, cut tissue into small pieces in a 1.5 ml tube and add 250 µl of the **RLB** (RNA Lysis Buffer) Disaggregate tissue pieces with a pestle by 10-20 strokes. Incubate at room temperature for 5 min, followed by incubating at 4°C for an additional 5 min.

3. RNA Isolation

3.1 After incubation, centrifuge the lysate at 14,000 rpm for 2 min. Then transfer the supernatant to a new 1.5 ml RNase-free tube. Add 200 µl of isopropanol and mix the sample by inverting the tube gently.

3.2 Place a F-spin column into a 2 ml collection tube. Transfer the mixture to a F-spin column. Centrifuge at 14,000 rpm for 30 sec. Remove the column from the collection tube and discard the flow through.

3.3 Replace column to the collection tube. Add 200 µl of working **RWB** (RNA Wash Buffer) to the column and centrifuge at 14,000 rpm for 30 sec. Discard the flow through.

3.4 Add 20 µl of **DNase I** working solution directly to the column filter. Incubate at room temperature for 10 min.

4. RNA Purification

4.1 Add 300 µl of working **RPB** (RNA Purification Buffer) to the column. Incubate at room temperature for 1 min and centrifuge at 14,000 rpm for 30 sec. Discard the flow through.

4.2 Add 200 µl of working **RWB** (RNA Wash Buffer) to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow through. Repeat step 4.2 once more.

5. Elution

Place the column in a new 1.5 ml tube (RNase-free). Add 15 µl of **EB** (Elution Buffer) directly to the column filter. Centrifuge at 14,000 rpm for 1 min to elute purified RNA.

Note*: For high concentrated RNA, use 8 µl to elute.*

Purified RNA is now ready for use or storage at -20°C for up to 2 months and at -80°C for long term.

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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 tubes.
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 **RLB** (RNA Lysis Buffer) to achieve the optimal lysis.
	Incorrect elution conditions.	Add 8-15 µl of the **EB** (Elution Buffer) to the center of each **Column**, let it stand for 2 minutes, and centrifuge at 14,000 rpm for 2 minutes.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA.	Repeat the wash step: Centrifuge at 14,000 rpm again for 2 minutes to remove the residual **RWB** (RNA Wash Buffer).

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)
P-9013	Epigenase™ m6A Demethylase Activity/Inhibition Assay Kit
P-9015	MethylFlash™ Urine N6-methyladenosine (m6A) Quantification Kit (Colorimetric)
P-9016	EpiQuik™ CUT&RUN m6A-Seq Kit
P-9018	EpiQuik™ CUT&RUN m6A Enrichment Kit
P-9106	EpiQuik™ Magbeads Quick RNA Isolation Kit

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