

Manual

QuickExtract RNA Extraction Kit

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QuickExtract™ RNA Extraction Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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1. Introduction

The QuickExtract RNA Extraction Kit is a fast, simple and inexpensive method for preparing RNA for RT-PCR amplification. The QuickExtract RNA Extraction Kit is a single-tube system that requires only vortex mixing to lyse the cells, and prepare the RNA for cDNA synthesis. This allows easy processing of one to hundreds of samples in minutes, with no sample loss and without the use of toxic organic solvents. The QuickExtract RNA Extraction Solution works with adherent and suspension cells and has been tested on human, mouse, rat and *E. coli* cell cultures. The RNA extracts have been used successfully for both end-point and real-time RT-PCR. An optional DNase I treatment may improve amplification performance by removing cellular DNA that may cause false-positive results.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
QuickExtract RNA Extraction Kit	50 mL (500 extractions)	QER090150	DNase I RNase-Free (1 U/ μ L)	E0013-1D2	2.5 mL
			RiboGuard™ RNase Inhibitor	E0126-40D4	1.25 mL
			Stop Solution	SS000664-D1	2 mL
			10X DNase I Reaction Buffer	SS000751-D2	5.5 mL
			QuickExtract RNA Extraction Solution	SS000880-D2	50 mL

3. Product specifications

Storage: Store the kit at -20 °C in a freezer without a defrost cycle.

Storage buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM CaCl₂ and 10 mM MgCl₂.

Unit definition: One Molecular Biology Unit (MBU) of RNase-Free DNase I converts 1 μ g of pUC19 DNA into oligodeoxynucleotides in 10 minutes at 37 °C.

Quality control: The QuickExtract RNA Extraction Kit is function-tested by assaying for a PCR product from cDNA produced from RNA extracted from HeLa cells.

Contaminating activity assays: All components of the QuickExtract RNA Extraction Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

4. Notes on use of the QuickExtract RNA Extraction Kit

1. Perform QuickExtract RNA extractions in an RNase-free work environment.
 - Always wear gloves when handling samples containing RNA and kit components.
 - Do not pick up any kit component with an ungloved hand.
 - Keep all kit components tightly sealed when not in use.
 - Keep all tubes containing RNA tightly sealed during the incubation steps.
2. The extraction protocol is scaleable. The reagent volume can be adjusted according to the number of cells being lysed.

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3. Lysates should be kept on ice when in use and when possible, used directly in cDNA synthesis reactions. Unused lysates should be stored frozen at -70 °C.

5. Protocols

A. Adherent or suspension cultured cells (10³ to 10⁶ cells)

1. Harvest the cells using a method appropriate to the cell type and growth vessel. If trypsin is used for adherent cells harvest, it should be inactivated.
2. Count the cells. Optional: Wash cells with PBS or similar buffer to remove serum and media components.
3. Pellet the cells by centrifugation at 1,000 x g at 4 °C for 5 minutes. Carefully aspirate the supernatant.
4. Add 100 µL of ice cold QuickExtract RNA Extraction Solution to the cells and vortex mix for 1 full minute.
5. The RNA can be used directly in a cDNA synthesis reaction or stored frozen at -70 °C.
6. The DNA can be removed with an optional DNase I treatment (see Part B).

B. Optional DNase I treatment

1. Important! Preheat the lysate at 65 °C for 2 minutes in a thermal cycler before proceeding.
2. Add 11 µL of DNase Buffer 1, 2.5 µL of RiboGuard™ RNase Inhibitor and 5 µL of RNase- free DNase I to the extracted RNA. Mix the sample and incubate the tube at 37 °C for 15 minutes.
3. Add 4 µL of Stop Solution to the tube, mix well and incubate at 65 °C for 10 minutes.
4. Store the tubes on ice or frozen until the sample is to be used for cDNA synthesis.

PCR amplification recommendations for cDNA and DNA

1. For cDNA synthesis, 1-10 µL of extracted RNA can be used directly with any reverse transcriptase and a standard 20 µL protocol. Up to 50% of the reaction volume can be extracted RNA.
2. For standard and fast end-point PCR cycling profiles, use 1-5 µL of cDNA.
3. When using extracts in real-time RT-PCR with SYBR® Green I Dye (Molecular Probes, Inc.), or other similar dye detection, DNase treatment of the extract before cDNA synthesis is recommended to remove DNA that can cause false-positive PCR results. All of the reagents required for DNase I treatment are included in the kit.
4. If possible, design primers so that DNase I treatment is not needed. Primers can be designed to prevent amplification of genomic RNA by choosing sequences that flank a long intron. Short cycling times are then used so that the DNA amplicon will not be produced while the shorter cDNA amplicon is efficiently produced. Primers can also be chosen that anneal to exon-exon boundaries in the cDNA. These sequences do not exist in the genomic DNA.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.



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