EpiNext™ Hi-Fi cDNA Synthesis Kit

Base Catalog # P-9004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ Hi-Fi cDNA Synthesis Kit is optimized to synthesize a DNA copy specifically using bisulfite-converted RNA and enriched RNA fragments. The kit is also suitable for cDNA synthesis using total RNA. The synthesized DNA can then be used for a variety of downstream applications including PCR and cDNA library construction. The RT enzyme included in the kit has low intrinsic RNase activity, which delivers the highest reverse transcription accuracy currently available and promotes full-length cDNA synthesis.

Starting Material and Input RNA Amount: RNA isolated from various tissue or cell samples can be used as starting material. The amount of RNA for each reaction can be 0.1 ng-2 µg. For optimal reaction, the input RNA amount should be 200-500 ng.

Precautions: To avoid cross-contamination, the following precautions are necessary for handling tube/vials: Carefully pipette the sample or solution into the tubes/vials. Use 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.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

Component	20 reactions Cat. #P-9004-20	Storage Upon Receipt
5X RT Reaction Buffer	100 µl	-20ºC
10 mM dNTP Mix*	25 µl	-20ºC
0.1M DTT*	50 µl	-20ºC
RNase Inhibitor*	25 µl	-20ºC
Random Primer (50 µM)*	25 µl	-20ºC
RT Enzyme Mix*	25 µl	-20ºC

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C. Upon receipt, store all of the components at -20°C immediately. The kit can be stable for up to 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Thermocycler without heated lid
- ☐ Pipette and pipette tips
- ☐ 0.2 ml PCR tubes
- ☐ 1.5 ml microcentrifuge tubes
- ☐ RNA sample

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ Hi-Fi cDNA Synthesis 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 call 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 EpiNext™ Hi-Fi cDNA Synthesis Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ Hi-Fi cDNA Synthesis Kit and methods of use contain proprietary technologies by EpigenTek.

DESCRIPTION

Effective and efficient preparation of cDNA from RNA can help ensure reliable data in various downstream applications including real-time qPCR and RNA/cDNA-sequencing. The EpiNext™ Hi-Fi cDNA Synthesis Kit has been optimized and validated for cDNA synthesis from various RNA samples, particularly from bisulfite-converted RNA and enriched RNA fragments. The kit contains all necessary components including a highly sensitive and thermostable recombinant reverse transcriptase which provides enhanced cDNA synthesis efficiency, high fidelity, and a wide range of activity for varying amounts of RNA templates (0.1 ng to 2 µg). Due to its high thermal stability, low RNase activity and resistance to inhibition by rRNA and tRNA, the recombinant RT included in the kit can be used for synthesis of long cDNA products at temperatures from 42°C-60°C using total RNA, and GC-rich templates such as bisulfite converted RNA. A recombinant RNase inhibitor is also included in the kit to prevent the degradation of target RNA due to ribonuclease contamination.

ASSAY PROTOCOL

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

1. Starting Materials

Input RNA Amount: RNA amount can range from 0.1 ng to 2 µg per reaction. An optimal amount is 200–500 ng per reaction. Starting RNA may be in water or in a buffer such as TE. RNA should be high quality and relatively free of DNA. DNAse I can be used to remove DNA and RNA should be eluted in RNase-free water.

RNA Storage: RNA should be stored at -20°C or -80°C until use.

2. cDNA Synthesis

a. Add the following in a 0.2-ml PCR tube on ice:

Component	Amount
RNA (200-500 ng)	10 µl
Random Primer (50 uM)	1 µl
10 mM dNTP Mix	1 µl

b. Heat in a thermocycler (no heated lid) at 65°C for 3 minutes, Place on ice immediately for at least 1 minute.

c. Add the following to the tube on ice:

Component	Amount
5X RT reaction buffer	4 µl
0.1M DTT	2 µl
RNase inhibitor	1 µl
RT enzyme mix	1 µl
Total Volume in tube	**20 µl**

Vortex the sample briefly to mix and collect by centrifugation. Incubate as follows: 42˚C for 45 min followed by 80˚C for 5 min (no heated lid).

Store the cDNA synthesis reaction at -20°C, or proceed directly to next application such as qPCR (see the Appendix "Working with qPCR") or RNA/cDNA-sequencing.

APPENDIX

Working With qPCR

When working with qPCR, we recommend using the EpiQuik™ Quantitative PCR Fast Kit (Cat # P-1029) which contains a hot start polymerase system and has been optimized to decrease the overall qPCR amplification time. The master mix is provided at 2X concentration for easier preparation of PCR reactions requiring only the addition of primers and templates. With this kit, the qPCR can be finished in as short as 70 min.

Prepare the PCR Reactions

Component	Size (µl)	Final Concentration
Master Mix (2X)	10 µl	1X
Forward Primer	1 µl	0.4-0.5 µM
Reverse Primer	1 µl	0.4-0.5 µM
cDNA Template	1-2 µl	50 pg-0.1 µg
RNase-free H_2O	6-7 µl	
Total Volume	**20 µl**	

For the negative control, use RNase-free water instead of cDNA template.

Program the PCR Reactions

Cycle Step	Temp	Time	Cycle
Activation	95°C	7 min	1
Cycling	95°C 55°C 72°C	10 sec 10 sec 8 sec	40-45
Final Extension	72°C	1 min	1

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
Little or no cDNA synthesis	Poor RNA quality (RNA is severely degraded).	Check if the sample RNA 260/280 ratio is between 1.9-2.0. Analyze RNA on a denaturaing gel to verify RNA integrity.
	RT inhibitor is contained in RNA.	The common RNA inhibitors such as SDS, EDTA and formamide can be removed by re-precipitation and clean-up of RNA with ethanol.
	Temperature is incorrect.	Check if the temperature is appropriate for cDNA synthesis
	Insufficient starting RNA amount	Increase the amount of starting RNA, especially for amplifying low-copy genes from total RNA.
	Kit is not stored or handled properly.	Store all components of the kit at -20°C
Poor specificity in qPCR	Non-specific primers.	PCR primers were not appropriate or were incorrectly designed. Ensure the primers are specific for the target genes.
	Genome DNA contamination.	Treat RNA with DNase I and re-purify.

RELATED PRODUCTS

RNA Bisulfite Modification

P-9003 Methylamp™ RNA Bisulfite Conversion Kit

PCR Analysis

P-1028 Methylamp™ MS-qPCR Fast Kit
P-1029 EpiQuik™ Quantitative PCR Fast Kit