EZscript All-in-one Reverse Transcription Kit (with DNase)

Catalog No.: RT3P

Description

The ***EZ*Bioscience**® EZscript All-in-one Reverse Transcription Kit (with DNase) is a new generation of rapid reverse transcription kit for genomic DNA removal and reverse transcription reaction in one step. Compared with the ***EZ*Bioscience**® *4× EZscript Reverse Transcription Mix II (with gDNA Remover) (Cat. No.: EZB-RT2G)*, this kit does not require a separate genomic DNA removal reaction, which is more convenient and can effectively reduce the risk of contamination and RNA degradation caused by complex sampling process. The kit mainly includes five tubes of reagents: Enzyme Mix, 5× EZscript All-in-one RT Buffer, NC Buffer, Oligo dT18 (20×) and Random Hexamer (20×). Among them, The Enzyme Mix contains DNase, RNase Inhibitor and reverse transcriptase; The 5× EZscript All-in-one RT Buffer contains buffer, dNTPs, etc; Oligo dT18 (20×) and Random Hexamer (20×) are packaged separately, which is convenient for adding gene-specific primers as needed.

The reverse transcriptase is a genetic engineered enzyme based on M-MLV (RNase H-) reverse transcriptase. And the multiple site-mutations of reverse transcriptase can obviously increase its affinity to RNA templates and its strand extending ability, which make reverse transcription reaction more efficient. Moreover, this transcriptase is rather resistant to common reverse transcriptase inhibitors. At the same time, the kit uses the latest optimized reaction system to further improve the reverse transcription efficiency. The reverse transcription reaction products are recommended for PCR, qPCR and gene cloning.

Components

Components	RT3P (100 Rxns)	RT3P-L (500 Rxns)
Enzyme Mix	200 µl	200 µl × 5 tubes
5× EZscript All-in-one RT Buffer	400 µl	400 µl × 5 tubes
NC Buffer	20 µl	20 µl × 5 tubes
Oligo dT18 (20×)	110 µl	110 µl × 5 tubes
Random Hexamer (20×)	110 µl	110 µl × 5 tubes
ddH_2O ($Nuclease^-$)	1 ml	1 ml × 5 tubes

Storage

Store at -20°C.

Caution

Avoid RNase contamination

Please keep the environment of experiment clean. Clean gloves and mask should be worn during the experiment. Centrifuge tubes, tips and other supplies used in the experiment must be RNase-free.

Protocol

Thaw template RNA on ice. Thaw Enzyme Mix, 5× EZscript All-in-one RT Buffer, NC Buffer, ddH_2O ($Nuclease^-$), Oligo dT18 (20×), Random Hexamer (20×) or gene-specific primer at room temperature (15 ~ 25°C) or on ice.

Mix each solution by inverting the tubes up and down 10 times. Centrifuge briefly to collect residual liquid from the sides of the tubes, and then put on ice for later use.

1. Set up reactions

1A. If used for reverse transcription reaction of mRNA/ circRNA/ lncRNA:

(1) Prepare the one-step reaction for genomic DNA elimination and reverse transcription on ice according to the following table.

Add 1 µg total RNA (10 pg ~ 2 µg adjustable) or 200 ng poly(A) mRNA (10 pg ~ 500 ng adjustable) to a sterile, Nuclease-free tube, then add 2 µl of Enzyme Mix, 4 µl of 5× EZscript All-in-one RT Buffer, 1 µl of Oligo dT18 (20×), 1 µl of Random Hexamer (20×), and then add ddH_2O ($Nuclease^-$) to up to 20 µl:

Components	Volume (20 µl)
total RNA *or* poly(A) mRNA	1 µg 200 ng
Enzyme Mix	2 µl
5× EZscript All-in-one RT Buffer	4 µl
Oligo dT18 (20×)	1 µl
Random Hexamer (20×)	1 µl
ddH_2O ($Nuclease^-$)	up to 20 µl

Note: When performing circRNA reverse transcription, Oligo dT18 (20×) can be used or not.

(2) (Optional) Prepare No Reverse-Transcriptase Control reaction.

No Reverse-Transcriptase Control is a negative control reaction without reverse transcriptase, which is used to detect whether there is genomic DNA residue in RNA template.

Add 1 μg total RNA (10 pg ~ 2 μg adjustable) or 200 ng poly(A) mRNA (10 pg ~ 500 ng adjustable) to a sterile, Nuclease-free tube, then add 4 μl of 5× EZscript All-in-one RT Buffer, 2 μl of NC Buffer, 1 μl of Oligo dT18 (20×), 1 μl of Random Hexamer (20×), and then add ddH_2O ($Nuclease^-$) to up to 20 μl:

Components	Volume (20 μl)
total RNA *or* poly(A) mRNA	1 μg 200 ng
5× EZscript All-in-one RT Buffer	4 μl
NC Buffer	2 μl
Oligo dT18 (20×)	1 μl
Random Hexamer (20×)	1 μl
ddH_2O ($Nuclease^-$)	up to 20 μl

1B. If used for reverse transcription reaction of target specific gene:

(1) Prepare the one-step reaction for genomic DNA elimination and reverse transcription on ice according to the following table.

Add 1 μg total RNA (10 pg ~ 2 μg adjustable) or 200 ng poly(A) mRNA (10 pg ~ 500 ng adjustable) to a sterile, Nuclease-free tube, then add 2 μl of Enzyme Mix, 4 μl of 5× EZscript All-in-one RT Buffer, 1 μl of gene-specific primer (2 μM), and then add ddH_2O ($Nuclease^-$) to up to 20 μl:

Components	Volume (20 μl)
total RNA *or* poly(A) mRNA	1 μg 200 ng
Enzyme Mix	2 μl
5× EZscript All-in-one RT Buffer	4 μl
gene-specific primer (2 μM)	1 μl
ddH_2O ($Nuclease^-$)	up to 20 μl

(2) (Optional) Prepare No Reverse-Transcriptase Control reaction.

Add 1 μg total RNA (10 pg ~ 2 μg adjustable) or 200 ng poly(A) mRNA (10 pg ~ 500 ng adjustable) to a sterile, Nuclease-free tube, then add 4 μl of 5× EZscript All-in-one RT Buffer, 2 μl of NC Buffer, 1 μl of Oligo dT18 (20×), 1 μl of Random Hexamer (20×), and then add ddH_2O ($Nuclease^-$) to up to 20 μl:

Components	Volume (20 μl)
total RNA *or* poly(A) mRNA	1 μg 200 ng
5× EZscript All-in-one RT Buffer	4 μl
NC Buffer	2 μl
gene-specific primer (2 μM)	1 μl
ddH_2O ($Nuclease^-$)	up to 20 μl

2. After the reaction is prepared according to steps 1A and 1B, mix thoroughly before the reverse transcription reaction (Important). It is recommended to adjust the volume of the pipette to about 18 μl and gently pipette 10 ~ 15 times.

3. Incubate at 42°C for 15 min, then Incubate at 95°C for 30 s to inactivate DNase and reverse transcriptase.

4. The reverse transcription reaction product can be directly used in PCR applications, but the cDNA is recommended to dilute and mix thoroughly before use (The specific dilution factor depends on the abundance of gene expression. Generally, cDNA is diluted 5 ~ 10 times). If the qPCR experiment is not performed immediately, it is recommended to store at -80°C for long-term storage. Avoid repeated freeze-thaw cycles.