EnergicScript® First Strand cDNA Synthesis Kits
for qPCR
User Manual

Cat.Nos.ZK00804(50 rxns×20ul)

ZK00805(100 rxns×20ul)

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Ⅰ、Description

The EnergicScript® cDNA Synthesis Kit includes all the necessary reagents for cDNA synthesis to be used in qPCR. Either total RNA, mRNA, viral RNA or *in vitro* transcribed RNA can be used as a template for reverse transcription. The kit includes both random primers and oligo(dT) primers. The user can choose either of these or lternatively use gene specific primers. Quantitative PCR (qPCR) is a useful technique for the investigation of gene expression,viral load, pathogen detection, and numerous other applications. When analyzing gene expression or viral load, the RNA of interest first needs to be reverse transcribed into cDNA. The EnergicScript® cDNA Synthesis Kit is intended for cDNA synthesis for two-step quantitative reverse transcription-PCR (qRT-PCR) applications, where amplicons are usually around 200 bp in length. This kit is can be used in conjunction with ShineProbe qPCR Kits or with any other qPCR kit suitable for the application. Gene pecific primers, random primer or oligo(dT) primers can be used for the RT step.Using specific primers can decrease background, whereas random primer and oligo(dT) primers are useful if several different amplicons need to be analyzed from a small amount of starting material.

1. random primer: at non-specific points along an RNA template. In this case, all RNA in a population are templates for cDNA synthesis.
2. oligo(dT)18: at the 3'-end of poly(A)+ mRNA. In this case, only mRNA with 3'-end poly(A) tails are templates for cDNA synthesis.
3. gene specific primer: at a primer-binding site.

Ⅱ、Importants Notes

1. Multiple freeze/thaw of RNA should be avoided. Thaw and keep control RNA on ice.
2. It is recommended that the first strand cDNA synthesis is carried out under conditions where Rnase contamination has been eliminated. Pipette tips and tubes should be treated with 0.1% diethylpyrocarbonate (DEPC) (soak overnight in 0.1% aqueous solution of DEPC at 37℃, then heat at 100℃ for 30 min and autoclave).
3. Wearing gloves is highly recommended.
4. As most users do not need to do control First Strand cDNA synthesis, this kit does not include control RNA.
5. Incubation at 40° C will work for most templates, but it can be optimized between 40° C
and 48° C if necessary. Raising the temperature can be helpful if the template has strong secondary structures. Higher temperature can also improve specificity if gene-specific primers are used. Incubation time of 30 min is sufficient in most cases. If the target is located near the 5' end of a long transcript and oligo(dT) priming is used, or the target is rare, cDNA synthesis time can be extended up to 60 min.
6. A separate RNA denaturation step is generally not required, but it can be performed before cDNA synthesis if the template RNA has a high degree of secondary structure. The denaturation step, 5 min at 65° C, should be performed before adding 2x RT buffer and

RTase to the reaction mix.

Ⅲ、Component

Components	ZK00804 (50 rnsX 20ul each)	ZK00805 (100 rnx X 20ul each)
2× RT Buffer	500ul	1000ul
random primer	50ul	100ul
oligo(dT)18	50ul	100ul
RTase	50ul	100ul
DEPC-ddH2O	1ml	1ml

Ⅳ、Shipping and Storage

The EnergicScript Kit is shipped in gel ice or ice bag. Upon arrival, store all kit components at -20°C. When using the kit, the leftover thawed mix can be refrozen and stored at -20 °C without affecting the performance of the kit. The kit is stable for six months from the date of packaging when stored and handled properly.

Ⅴ、Reaction Setup

Components	Volume(μl)	Comments
2× RT Buffer	10	Mix thoroughly
random primer	1	Alternatively oligo(dT) or a specific primer can be used.
RTase	1	Includes RNase inhibitor
RNA(ul)	X	Max 1μg,usually 2-6ul
DEPC -ddH_2O (ul)	Y	Add water to fill up to the final reaction volume
Total Volume(ul)	20	

Ⅵ、Cycling Protocol

1、Protocol using random primer

25℃10min→40℃30 min→80℃2 min→4℃ hold

2、Protocol using oligo(dT)18

25℃10min→42℃30 min→80℃2 min→4℃ hold

3、Protocol using specific gene primer

48℃30 min→80℃2 min→4℃ hold

Ⅶ、Reference

1. Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A Laboratory Manual (2nd Ed.) Cold Spring Harbor University Press, Cold spring Harbor, NY, 1989.
2. Ausubel, F.M. et al. eds., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, 1997.

Ⅷ、Technical Supports

To access the ShineGene Web site, go to:**http://www.shinegene.org.cn**

The ShineGene Web site provides a list of telephone,Email and fax numbers that can be used to contact Technical Support. E-mail:master@shinegene.org.cn

Ⅸ、Related Products

ShinePrep® RNA Miniprep Kits
Cat.Nos.ZN00701(50 rxns)
ZN00702(100 rxns)

ShineProbe® Real Time qPCR Kits
Cat.Nos.ZK00713(50 rxns×50ul)
ZK00714(100 rxns×50ul)

FicoScript® M MLV Reverse Transcriptase
Cat.Nos.ZP00601(1000 U)
ZP00602(5000 U)

ShinePolo® One step RT-PCR qPCR Kits
Cat.Nos.ZK00102(50 rxns×30ul)
ZK00103(100 rxns×30ul)

PowerQ® Taq plymerase
Cat.Nos.ZP00102(1000 U)
ZP00103(5000 U)

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