Tinzyme Co., Limited

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RT Script One Step RT-PCR Kit

Product Number: PCK34

Shipping and Storage

Stored at -20℃, valid for 12 months.

Component

Component	100T
2×Script OneStep RT-PCR Buffer	1mL
Script OneStep Enzyme Mix	100μL
RNase free Water	1mL

Description

This kit is specifically designed for one-step RT-PCR experiments, and can be used to conveniently and quickly complete reverse transcription and PCR amplification reactions in the same reaction tube. During the reaction process, there is no need to open the tube cap to add reagents, which avoids contamination while improving detection sensitivity and experimental efficiency. This kit includes the OneStep Enzyme Mix optimized for optimal ratio (including mutated TRUEscript M-MuLV H Minus reverse transcriptase, hot start HotMaster Taq DNA polymerase, and RNasin inhibitor mix), as well as a unique 2 × RT-PCR Mix reaction system suitable for reverse transcription and PCR amplification. The RNase H activity of this enzyme M-MuLV (RNase H $^-$) is deficient. Compared with M-MuLV, it has stronger extensibility and stability, and can be used for longer cDNA synthesis and the construction of high proportion full-length cDNA libraries. At the same time, the enzyme enhances heat resistance and can reverse transcribe at 42-50 ° C, improving the efficiency of complex secondary structures and GC rich template reverse transcription.

Application

Suitable for high copy and low copy gene testing; RNA templates with high GC content or complex secondary structures.

Protocol

1. Suggested reaction system (20μL-50μL)

Note: Before using the 2×Script OneStep RT-PCR Buffer, thoroughly melt and mix it upside down to avoid the generation of large amounts of bubbles due to violent vortices. Short term frequent use can be stored in a refrigerator at 4℃.

Prepare the reaction solution on ice according to the table below:

Component	Volume	Final Concentration
2×Script OneStep RT-PCR Buffer	10μL	1×
Forward Primer（10μm）	0.4μL	0.2μM
Reverse Primer（10μm）	0.4μL	0.2μM
Script OneStep Enzyme Mix	1μL	-
RNA template	XμL	0.1-2μg
RNase Free Water	To 20μL	Not applicable

Note: Please use the final concentration of 0.2-0.6μM as a reference for setting the primer concentration range. In the case of low amplification efficiency, the concentration of primers can be increased; When non-specific reactions occur, the primer concentration can be reduced to optimize the reaction system. If multiple reactions are carried out simultaneously, first prepare a mixture according to the proportion, shake and mix well, and then divide it into 20-XμL(X is the template amount) per tube.

2. PCR amplification

2.1. Mix and centrifuge the prepared reaction system.

2.2. Preheat the thermal cycler to 50℃, place the PCR tube in the thermal cycler, and perform the reaction under the

following conditions. Amplification program.

	Temperature	Time	Cyclic number
1	50℃	30min	1
2	94℃	2-3min	1
3	94℃	30sec	30-40
	50-60℃	30sec	
	72℃	1-2kb/min	
4	72℃	5-10min	1
5	4℃	Insulation	

Note: The number of cycles can be set based on the downstream application of the amplified product. Too few cycles and insufficient amplification; Multiple cycles increase the probability of mismatches and result in severe non-specific background. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.

Note

1. Avoid RNase contamination.
2. To ensure a successful reaction, it is recommended to use high-quality RNA templates.
3. Different fragments require different optimal amounts of RNA templates, and excessive RNA can inhibit the reaction. It is recommended to adjust the template amount according to the reaction.
4. Oligo (dT) and Random Primer cannot be used to synthesize cDNA.