CYPC34-TRUEscript One Step RT-PCR Kit

❖ Kit Contents and Storage

Product component	Size	
	CYPC3401	CYPC3402
	125 rxns × 20μl system	250 rxns × 20μl system
2 × RT-PCR Mix	1.25ml	2 × 1.25ml
TRUEscript OneStep Enzyme Mix	100μl	200μl
RNase free H_2O	1.5ml	1.5ml

Store at -20°C. Stable for 6 months.

Transportation and Storage

Transport on blue ice and store at -20°C.

Product Description

This kit is designed for one-step RT-PCR experiments. It allows you to perform the reverse transcription and PCR amplification reactions conveniently and quickly in the same tube. No need to open the tube cap to add reagents during the reaction process, which avoids contamination and increases the sensitivity and efficiency of the test. This kit includes the optimally proportioned OneStep Enzyme Mix (containing the mutation-modified TRUEscript M-MuLV H Minus reverse transcriptase, the hot-start HotMaster Taq DNA polymerase and the 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 the enzyme M-MuLV (RNase H-) is missing. Compared with M-MuLV, it has stronger extension ability and stability. It can be used for longer cDNA synthesis and construction of a high proportion of full-length cDNA libraries, etc. At the same time, the enzyme improves heat resistance and can perform reverse transcription reactions at 42-50°C, effectively improving the reverse transcription efficiency of complex secondary structure, GC-rich templates.

Product Features

The product is suitable for high-copy, low-copy gene detection, as well as some RNA

templates with high GC content or with complex secondary structures.

Recommended reaction system (20μl-50μl reaction system)

Note: Please thaw and mix gently before using the 2×RT-PCR Mix (to avoid a lot of air bubbles from violent swirling). Stored at 4°C for short-term, frequent use.

Prepare the reaction system on ice according to the following table.

Components	Volume	Final Concentration
2×RT-PCR Mix	10 μl	1×
Forward Primer (10 μM)	0.4 μl	0.2 μM
Reverse Primer (10 μM)	0.4 μl	0.2 μM
TRUEscript Enzyme Mix	0.8 μl	-
RNA template	X μl	1 pg–1 μg
RNase-free H_2O to final volume	20 μl	Not applicable

Note: For the primer concentration, please use the final concentration of 0.2-0.6 μM as the reference for the setting range. In case of low amplification efficiency, the primer concentration can be increased; in case of non-specific reactions, the primer concentration can be reduced to optimize the reaction system.

PCR Amplification

1. Mix and centrifuge the prepared reaction system.
2. Preheat the PCR instrument to 42°C, place the PCR tube containing the prepared reaction solution in the PCR instrument and perform the reaction according to the following reaction conditions.

Amplification Condition

Steps	Temperature	Time	Cycle Number
1	42°C	30 min	1
2	94°C	2-3 min	1
3	94°C	30 sec	30-40
	50-60°C	30 sec	
	72°C	1-2kb/min	
4	72°C	5-10 min	1
5	42°C	Keep warm	

Note: The number of cycles can be set based on the downstream application of the amplification

product. Too few cycles result in insufficient amplification; too many cycles increase the chance of mismatch and non-specific background. Therefore, the number of cycles should be kept to a minimum while ensuring the yield.

Notes

a. Avoidance of RNase contamination.

b. To ensure the success of the reaction, the use of high-quality RNA templates is recommended.

c. Different fragments require different amounts of optimal RNA templates, too much RNA can inhibit the reaction, and it is recommended to adjust the RNA template amount according to the actual reaction.

d. Cannot use Oligo (dT) and Random Primer to synthesize cDNA.

This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.