

HelixCRIPT™ One-Step RT-PCR Kit [*Hot-Taq*] [UDG System]

Kit Contents

HelixCRIPT™ One-Step RT-PCR Kit [<i>Hot-Taq</i>] [UDG System]	
Cat. No.	ORTHT2U100 (100rxns)
Enzyme Mix [<i>Hot-Taq</i>] (with UDG)	0.2ml x 1ea
2x Reaction Mix [<i>Hot-Taq</i>] (with dUTP)	1.25ml x 2ea
Instruction for Use	1ea

Description

HelixCRIPT™ One-Step RT-PCR Kit [*Hot-Taq*] [UDG System] is designed for sensitive amplification of the target gene in one-tube reaction from total transcripts. The Enzyme Mix contains in this kit, is an optimized blend of Reverse Transcriptase, HelixCRIPT™ *Thermo* Reverse Transcriptase and a HelixAmp™ *Hot-Taq* polymerase, RNase inhibitor protein and a thermolabile UDG. The UDG/dUTP system prevents the carryover contamination of PCR products from previous reactions.

HelixCRIPT™ One-Step RT-PCR Kit [*Hot-Taq*] [UDG System] : High specific and sensitive RT-PCR

One-step RT-PCR system provide the several advantages.

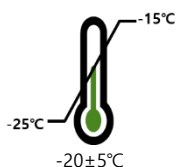
- Synthesis of cDNA and PCR amplification corresponding to target gene in one-tube reaction
- Obtain the reproductive data in the repetitive experiment(s)
- Can save the time and cost for preparation of RT-PCR
- Amplification of low-copy transcripts by RT-PCR

Application

Detection of target gene transcript from RNA

Semi-quantitative, quantitative analysis of RNA transcription level

Storage



Shelf life



NanoHelix Co., Ltd.

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Quality control assay data

Functional analysis

The activity for cDNA synthesis and PCR amplification of target gene transcript using HelixCript™ One-Step RT-PCR Kit [*Hot-Taq*] [UDG System] evaluated by Limit-of Detection (LOD) assay in human total transcripts.

Quality authorized by Yountaek Go



Protocol

1. Program the thermal cycler as follows in order to synthesize cDNA using HelixCript™ One-Step RT-PCR Kit [UDG System] with HelixAmp™ *Hot-Taq*.

Step	Condition		Cycle(s)
[Optional] UDG Activation ¹⁾		25°C for 5 min	1
cDNA Synthesis		42 ~ 55°C for 30 ~ 50 min	1
Pre-denaturation		95°C for 12 ~ 15 min	1
PCR Amplification	Denaturation	95°C for 20 sec	30 ~ 40
	Annealing	²⁾ AT°C for 40 sec	
	Extension	72°C for 1 min/kb	
Post Extension		72°C for 5 min	1

¹⁾ The UDG reaction step is not essential. The UDG will efficiently remove carryover contaminant DNA during sample setup and cycler ramping.
The reaction time for each steps should be optimized on the applied thermocycler.

²⁾ **AT**, annealing temperature of primers used

$$\text{Annealing Temperature} = T_m - (6 \sim 8^\circ\text{C})$$

$$\text{Where, } T_m \text{ (Melting Temp.)} = [4^\circ\text{C} \times (\text{G} + \text{C})] + [2^\circ\text{C} \times (\text{A} + \text{T})]$$

2. Add the following components into 0.2 or 0.5ml micro-tube.

Components	Volumes
RNA Template (1ng ~ 5μg)	X μl
Forward primer (10pmoles/μl)	2μl
Reverse primer (10pmoles/μl)	2μl
2x Reaction Mix [<i>Hot-Taq</i>] (with dUTP)	25μl
Enzyme Mix [<i>Hot-Taq</i>] (with UDG)	2μl
RNase-free Water	to 50μl

※ RNAs : Total RNA : 10ng ~ 5μg, Poly(A)⁺ RNA : 1ng ~ 500ng

3. Gently mix and immediately centrifuge the reaction mix.

4. Perform the one-step RT-PCR.

Products

Cat. No.	Products	Size
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