One-Step RT-PCR Kit

Catalog No.	50503S	50503L
Reactions	100 reactions	500 reactions

Description

One-Step RT-PCR Kit is a convenient and quick solution for performing RT-PCR in a single step. RT-PCR is a well-used technique to amplify double-stranded DNA fragments from RNA templates. In the first reverse transcription step, the reverse transcriptase synthesizes cDNA based on the RNA template. In the second PCR step, Taq DNA Polymerase synthesizes DNA using the synthesized cDNA as template.

The One-Step RT-PCR Kit combines reverse transcription and PCR into one reaction step, which eliminates the need for two-step reactions. The one-step strategy avoids the transfer of reaction mixtures from the reverse transcription step to the PCR step, and reduces the contamination risk and minimizes tedious sample handling.

The unique formulation of both buffer and enzyme mix delivers reliable and consistent results. The optimized buffer components allow for efficient reverse transcription and PCR with secondary structure and ensure complete cDNA synthesis and specificity of the PCR fragments.

The buffer of the One-Step RT-PCR Kit includes dNTPs, MgCl2, PCR enhancer, and PCR stabilizer. The enzyme mix includes M-MuLV reverse transcriptase, Taq DNA polymerase, enzyme enhancer, and enzyme stabilizer. M-MuLV Reverse Transcriptase is an RNA-directed DNA polymerase that can synthesize DNA strands using RNA as template. The source of the Reverse Transcriptase is a cloned reverse transcriptase gene from M-MuLV. Taq DNA Polymerase is a thermostable DNA polymerase that possesses a 5′→ 3′ polymerase activity and a low 5′→ 3′ exonuclease activity. The source of the Taq DNA Polymerase is a cloned Taq DNA Polymerase gene from Thermus aquaticus YT-1.

Features

- cDNA synthesis coupled with PCR in one step
- Robust and reliable reactions
- Unique buffer and enzyme formulations generate consistent results
- PCR fragments have 3'-dA overhangs

Component

Catalog No.	**50503S**	**50503L**
RP Buffer (5X)	400 µl	2000 ul
RP Enzyme	100 ul	500 ul
PCR Water	1500 ul	7500 ul

Storage Condition

- Store kit at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Thermal cycler
- PCR plate or PCR rubes
- PCR plate seal film

Protocol

Reaction setup

Suggested reaction setup (on ice recommended):

Component	Volume	Final conc.
RNA template*	variable	
RP Buffer (5X)	4.0 µl	1X
Forward Primer (10 µM)	0.8 µl	0.4 µM
Reverse Primer (10 µM)	0.8 µl	0.4 µM
RP Enzyme	1.0 µl	1X
Water	variable	
Total Volume	20 µl	

* RNA template between 1 µg and 1 pg can be used. The amount can be varied dependent on the expression levels of the targeted genes.

Reaction condition

Suggested condition RT-PCR:

Step	Temperature	Time	Cycles
Reverse Transcription	42°C	20 minutes**	1
Initial denaturation	94°C	60 seconds	1
Denaturation	94°C	30 seconds	25-35 cycles
Annealing	50-65°C***	30 seconds	
Extension	72°C	60 seconds****	
Final extension	72°C	2 minutes	1

**For the cDNA synthesis of long transcripts, extend the Reverse Transcription step up to 45 min.
***Optimal annealing temperature may vary based on primer design.
**** Adjust the extension time based on the amplicon size.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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