Ver.24111

OneStep RTPCR Kit

Cat.No. ODR61

Description: The OneStep RTPCR Kit contains all necessary reagents for both reverse transcription and PCR amplification, using gene-specific primers and target RNAs from either total RNA or mRNA.

Taq PCR Smart Mix (2X) is a proprietary mix of *Taq* DNA polymerase, Buffer, dNTPs and $MgCl_2$ for easy and convenient use. The product is recommended to use amplification less than 5kb PCR product.

Product contents

Contents	Volume
RTase (200U/µL)	5000U
5X RT Reaction Buffer (Included 50mM DTT mix)	200µL
10mM dNTP	100µL
RNase-free Water	600µL
Random hexamer (2.8nmol)	Lyophilized Dissolve in 56µL nuclease free water.
Oligo$(dT)_{18}$ (2.8nmol)	Lyophilized Dissolve in 56µL nuclease free water.
Taq PCR Smart Mix (2X) (without dye)	1mL

Note: Prepare aliquots of Random Hexamer and Oligo $(dT)_{18}$ to avoid multiple freeze thaw cycles.

Storage

- Store at –20°C

Features

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RNase H activity	RNase H negative
Temperature of cDNA synthesis	At 42-55ºC
Synthesis length	< 14 kb
Synthesis Primer	Random primer, Oligo $(dT)_{18}$, Gene specific

Protocol

- **First Strand cDNA synthesis (Total reaction volume - 20µL)**

1. Prepare the following mixture in a PCR tube.

RNA	10ng –1µg total RNA 10ng – 0.5µg mRNA	- µg
Primer	1µL Oligo $(dT)_{18}$ /1µL Random hexamer or 0.5µL Oligo $(dT)_{18}$ + 0.5µL Random hexamer or 1µL Gene-specific Primer (15 – 20µM)	
dNTP (10mM)		1µL
Make up the volume with RNase-free Water to		15.5µL

2. Heat the mixture at 65ºC for 5 minutes and cool down immediately on ice for 1 minute. Spin down the tube by centrifugation briefly.

   Note: Use heating block or PCR machine. DO NOT use water bath.

3. Add the following components to the same tube:
(Total reaction volume - 20µL)

Reagents	Volume
5X RT Reaction Buffer	4µL
RTase	0.5µL

4. Mix gently.
   Note: For random hexamer, incubate the combined reaction mixture at 25ºC for 5 minutes and then proceed with the next step. For oligo $(dT)_{18}$ or gene-specific primers, directly proceed with the next step.

5. Incubate at 50ºC for 10 minutes.

6. Inactivate the reaction mixture by heating at 95ºC for 5 minutes.

   Note: Use heating block or PCR machine. DO NOT use water bath.

7. The product can be used for PCR immediately or stored at -20ºC.

   Note: As a recommendation; cDNA product should compose 10% of total reaction volume of PCR.

- **PCR amplification using cDNA as template**

  Recommended PCR mixture and cycling condition

PCR mixture (Reaction vol. 50µL)		Cycle		
Taq PCR Smart Mix (2X)	25µL	95°C	2 min	×1
Forward primer (10pmol/µL)	2µL	95°C	20 sec	×35-40
Reverse primer (10pmol/µL)	2µL	AT	40 sec	
Template cDNA (<200ng)	-µL	72°C	1 min/kb	
Add D.W to	50µL	72°C	5 min	×1

Modifications on the amount of template, extension time, annealing temperature, and the number of PCR cycles can be done according to the target size, primer's Tm, and the type of templates for amplification.