Taq-Probe Polymerase

Type and Catalog Number

Taq-Probe Polymerase
Cat. # W145, 250 reactions x 20µL (No ROX)

Intended Use

• This DNA-dependent DNA polymerase is specially engineered for real-time PCR and RT-PCR amplification with TaqMan probe, singleplex or multiplex.

• It is used together with Thermophilic Reverse Transcriptate (Cat 140) for RT-PCR.

Characteristics

• *Taq*-Probe polymerase is specially engineered for TaqMan probe, its increased 5'-3' exoclease activity generating S-shaped curve.

Table 1. *Taq*-Probe polymerase properties

5'-3' polymerase activity	Yes
5'-3' exonuclease activity	Yes
3'-5' exonuclease activity	No
Reverse transcriptase activity	Neglectable
Incorporation of modified nucleotides	Yes, such as dUTP, fluorescence dye-labeled dNTPs
Terminal transferase activity	Minimal

Table 2. PCR and RT-PCR optimal parameters

Optimal RTase amount	Singleplex: 0.5-1U/20µl reaction
	Multiplex: 1-2U/20µl reaction
Optimal Taq-Probe polymerase amount	Singleplex: 2U/20µl reaction
	Multiplex up to four templates: 4U/20µl reaction
Optimal temperature	72-75°C
Heat inactivation	>96°C
dNTP concentration	Each 200uM
MgCl2 concentration	3mM
Primer concentration	Each ≥0.15µM, depending on primer design and thermocycling
TaqMan probe	Each 0.15-0.25µM
Template	RNA: as low as single digit copies of target RNA
	Human genomic DNA: ≤1000ng/20µl reaction
Product size	Preferably 75-150bp

Unit Definition

One unit of the enzyme catalyzes incorporation of 10nmol of deoxyribonucleotides into polynucleotide in 30 min.

Production Source

E. coli strain

Transportation and Storage

The kit can be stored at ≤-20°C for 24 months. The product can be transported below 4 °C for up to 3 days.

Table 3. Kit Contents

Content	Amount
Taq-Probe polymerase, 25U/µl	500U/2500U
5x *Taq*-Probe buffer C	2x1mL/10mL
User manual	1

Setup Reaction and Thermocycling

1. Thaw 5x Buffer and other reaction components at room temperature, mix each component, centrifuge, and then place on ice.

2. Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge, and then place on ice.

3. Aliquot the assay mix into PCR tubes or plates.

4. Add RNA template to PCR tubes or plate.

5. Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.

6. Mix and then briefly centrifuge the tubes or plate.

7. Program PCR instrument with indicated thermo-cycling protocol.

8. Load PCR tubes or plates and start to run.

9. Perform data analysis according to the PCR instrument instructions.

Table 4. Set up a 20µl of reaction

Content	Amount and final concentration
5x Buffer C	4µl
dNTPs	Each 200µM
Primers[a]	Each ≥0.15µM
TaqMan probe[b]	Each 0.15-0.25µM
Thermophilic Reverse Transcriptase[c]	Singleplex: 0.5-1U
	Multiplex: 1-2U
Taq-Probe polymerase	Singleplex: 2U
	Multiplex: 4U
Template	RNA: as low as single digit copies of target RNA
	Human genomic DNA: ≤1000ng/20µl reaction
H_2O	To 20µl

Footnotes of Table 4

[a] The primer T_m should be designed ≥60°C, preferably between 62°C to 65°C, using primer3 software for high efficiency and specificity.

[b] The probe's T_m should be designed between 70-75°C.

[c] Thermophilic Reverse Transcriptase, Cat RT-PCR 140, is not included.

Table 5. Compatible instruments

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Table 6. Standard thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	

Table 7. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	

Footnotes of Tables 6 and 7

The three-step thermocycling protocol in Table 7 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 6.

The primer concentration used in Tables 6 and 7 is typically 0.15-0.2uM.

Related Products

- Thermophilic Reverse Transcriptase, Cat. # W140
- *Taq*-Probe Polymerase, Cat. # W145
- *Taq*-Fast Polymerase, Cat. # W148
- 1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat. # W147
- 1-Step 2X RT-PCR Master Mix-TaqMan Probe, Cat. # W143
- 1-Step 2X Multiplex RT-PCR Master Mix-TaqMan Probe, Cat. # W146
- 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe, Cat. # W149