Research use only

Taq-Fast Polymerase

Type and Catalog Number

Taq-Fast polymerase, Cat RT-PCR 148, 500U/2500U for 500/2500 reactions x 20µL (No ROX, Low ROX or High ROX)

Intended Use

• This DNA-dependent DNA polymerase is used real-time PCR and RT-PCR amplification with SYBR Green dye.

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

Characteristics

• *Taq*-Fast polymerase extends more than 300 bases with short PCR cycling program.

• The 5x buffer has three formulations of ROX, Low ROX or High ROX concentrations for your choice.

Table 1. *Taq*-Fast 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

Thermophilic Reverse Transcriptase	0.5-1U/20µl reaction
Taq-Fast polymerase amount	1-1.5U/20µl reaction
Optimal temperature	72-75°C
Heat inactivation	>96°C
dNTP concentration	Each 200uM
$MgCl_2$ concentration	1.5mM
Primer concentration	Each ≥0.15µM, depending on primer design and thermocycling
Template	RNA: as low as single digit copies of target RNA Human genomic DNA: ≤60ng/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-Fast polymerase, 25U/µl	500U/2500U
5x *Taq*-Fast buffer **B**- SYBR Green dye	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 plate.

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 plate 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 *Taq*-Fast buffer **B**	4µl
dNTPs	Each 200µM
Primers[a]	Each ≥0.15µM
Thermophilic Reverse Transcriptase	0.5-1U
Taq-Fast polymerase	1-1.5U
Template	RNA: as low as single digit copies of target RNA
	Human genomic DNA: ≤60ng/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.

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	
IV	60°C to 95°C	Various	1

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.

Table 8. Fast thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60˚C	5-10min	1
II	95˚C	1min	1
III	95°C	5sec	35-40
	60°C, signal acquisition	30sec	
IV	60°C to 95°C	Various	1

Footnotes of Table 8

The product size for the fast thermocycling protocol is preferred to be less than 90bp.

The primer concentration used is typically between 0.4uM and 0.9uM.

Precautions

If you order a "**No ROX**" master mix but have an Applied Biosystems/ThermoFisher instrument, please **turn off ROX passive reference dye button** when setup assays.