PowerQ® Taq DNA Polymerase

for qPCR

User Manual

Cat.Nos.ZP00102(1000 U)

ZP00103(5000 U)

Published 24 Feb 2007

Storage condition: The undiluted enzyme solution is stable when stored at -20℃.

Storage and dilution buffer: 20mM Tris-HCl; 1mM dithiothreitol; 0.1mM EDTA; 0.1M KCl; Nonidet P40, 0.5%(v/v); Tween 20, 0.5%(v/v); glycerol, 50%(v/v); pH 8.0 (4℃).

Concentration:5U/ul

Unit definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10mmols of dNTP into an acid-insoluble material in 30 minutes at 74℃. The reaction conditions are: 50mM Tris-HCl, (pH 9.0 at 25 ℃), 50mM NaCl, 10Mm $MgCl_2$, 200uM dATP, dCTP, dGTP and radiolabelled dTTP, and 12.5ug activated calf thymus DNA in a 50ul reaction.

10×Reaction buffer（without Mg2+）: 100mM Tris-HCl; 500mM KCl; pH 9.0 (20℃). Buffer is optimized for use with 0.2mM for each of dNTPs. A tube of 25mM $MgCl_2$ is supplied separately.

Reaction Mixture Set Up for qPCR

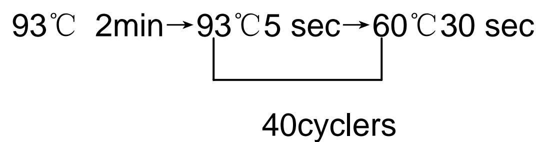
1.Gently vortex and briefly centrifuge all solutions after thawing.

2.Add, in a thin-walled PCR tube, on ice:

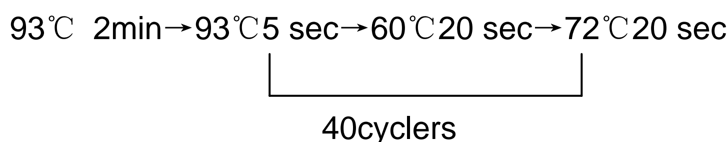
Reagent	Quantity, for 50μl of reaction mixture	Final concentration
Sterile deionized water	variable	-
10X *Taq* buffer	5μl	1X
10mM dNTP mix	1μl	0.2mM of each
25mM $MgCl_2$	7ul	3.0-4.0mM
Primer I	variable	0.4-1μM
Primer II	variable	0.4-1μM
Taqman probe	variable	0.2-0.3uM
Taq DNA Polymerase	0.5	2.5u / 50μl
Template DNA	variable	10pg-1μg
Total	50ul	

Cycling Protocol for qPCR

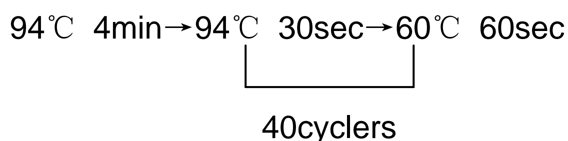
1、Protocol using LightCycler with Taqman probe



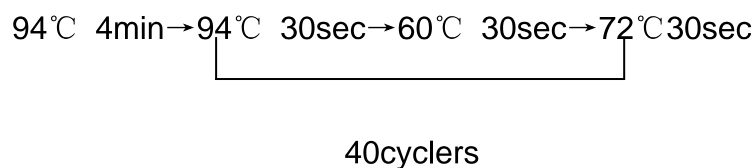
2、Protocol using LightCycler with Molecular Beacon probe



3、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Taqman probe



4、Protocol using other instruments, e.g. from Applied Biosystems, Bio-Rad Laboratories, Corbett Research, ,and Stratagene. with Molecular Beacon probe



Reaction Mixture Set Up for Electrophoresis PCR

1.Gently vortex and briefly centrifuge all solutions after thawing.

2.Add, in a thin-walled PCR tube, on ice:

Reagent	Quantity, for 50µl of reaction mixture	Final concentration
Sterile deionized water	variable	-
10X *Taq* buffer	5µl	1X
10mM dNTP mix	1µl	0.2mM of each
25mM $MgCl_2$	4ul	1.5-2.0mM
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
Taqman probe	variable	0.2-0.3uM
Taq DNA Polymerase	0.5	2.5u / 50µl
Template DNA	variable	10pg-1µg
Total	50ul	

4. Gently vortex the sample and briefly centrifuge to collect all drops from walls of tube.
5. If using a thermal cycler without a heated lid, overlay the sample with a half volume of mineral
oil or add an appropriate amount of wax.
6. Place samples in a thermal cycler preheated to 94°C and start PCR.

Recommended thermal cycling conditions for e-PCR:

Step	Temperature,°C	Time ,min	Number of cycles
Initial denaturation	94	4	1
Denaturation	94	0.5	
Annealing	50-68	0.5-1	25-40
Extension	72	0.5-2	
Final Extension	72	5	1

After cycling,maintain the reactions at 4°C or store at -20°C unil ready for analysis.

Note: It is recommended to add dNTPs to this incubation mixture shortly before use.

This is to prevent decomposition of the deoxynucleoside thiphosphate that occurs during Prolonged storage at the alkaline pH values required for optimal enzyme activity.

Quality control

Each lot of Taq DNA Polymerase is tested for activity in PCR and efficient incorporation of digoxigenin-11-dUTP, and in DNA sequencing of M13mp18ssDNA. A minimum of 250 bases must be clearly legible in the sequencing gel. Each lot of Taq DNA ploymerase is tested for contaminating activities as stated below.

Test buffer: 10mM Tris-HCl; 1.5mM $MgCl_2$; 50mM KCl; pH 9.0 (20℃). 0.1mg/ml gelatin.

Absence of endonucleases: 1ul lambda DNA is incubated with Taq Dna polymerase in 50ul test buffer with a paraffin oil overlay at 65℃ for 16 hours. The amount of enzyme showing no degradation of the lambda DNA is ststed under “Endol”.

Related Products

ShinePrep® RNA Miniprep Kits
Cat.Nos.ZN00701(50 rxns)
ZN00702(100 rxns)

ShineProbe® Real Time qPCR Kits
Cat.Nos.ZK00713(50 rxns×50ul)
ZK00714(100 rxns×50ul)

FicoScript® M MLV Reverse Transcriptase
Cat.Nos.ZP00601(1000 U)
ZP00602(5000 U)

ShinePolo® One step RT-PCR qPCR Kits
Cat.Nos.ZK00102(50 rxns×30ul)
ZK00103(100 rxns×30ul)

EnergicScript cDNA First Strand Synthesis Kit
Cat.Nos.ZK00804(50 rxns×20ul)
ZK00805(100 rxns×20ul)

Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add：Floor 2,Building A,328#, Wuhe Road,Shanghai,201109,China

Tel：+86-21-54460832

Fax:+86-21-54460831

E-mail:master@shinegene.org.cn

Website：www.synthesisgene.com