



PowerQ® Taq DNA Polymerase

for qPCR (Taq 酶)

User Manual

Cat.Nos.ZP00102(1000 U)

ZP00103(5000 U)

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

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°C).

Concentration: 5U/μl

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°C. The reaction conditions are: 50mM Tris-HCl, (pH 9.0 at 25 °C), 50mM NaCl, 10Mm MgCl₂, 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°C). Buffer is optimized for use with 0.2mM for each of dNTPs. A tube of 25mM MgCl₂ is supplied separately.

Reaction Mixture Set Up for qPCR

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

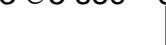
2. Add, in a thin-walled PCR tube, on ice:

| Reagent | Quantity, for 50μl of reaction mixture | Final concentration |
|----------------------------------|--|---------------------|
| Sterile deionized water | variable | - |
| 10X <i>Taq</i> buffer | 5μl | 1X |
| 10mM dNTP mix | 1μl | 0.2mM of each |
| 25mM MgCl ₂ | 7μl | 3.0-4.0mM |
| Primer I | variable | 0.4-1μM |
| Primer II | variable | 0.4-1μM |
| <i>Taqman probe/Sybr Green I</i> | variable | 0.2-0.3μM/0.2 X |
| <i>Taq</i> DNA Polymerase | 0.5 | 2.5u / 50μl |
| Template DNA | variable | 10pg-1μg |
| Total | 50μl | |

Cycling Protocol for qPCR

1、Protocol using LightCycler with Taqman probe

93°C 2min→93°C 5 sec→60°C 30 sec



40cyclers

2、Protocol using LightCycler with Molecular Beacon probe

93°C 2min→93°C 5 sec→60°C 20 sec→72°C 20 sec



40cyclers

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

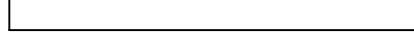
94°C 4min→94°C 30sec→60°C 60sec



40cyclers

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

94°C 4min→94°C 30sec→60°C 30sec→72°C 30sec



40cyclers

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 |
| 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.

* Table for selection of 25mM MgCl₂ solution volume:

| Final concentration of MgCl ₂ in 50 μl reaction mix(mM) | 1.0 | 1.25 | 1.5 | 1.75 | 2.0 | 2.5 | 3.0 | 4.0 |
|--|-----|------|-----|------|-----|-----|-----|-----|
| Volume of 25 mM MgCl ₂ (μl) | 2 | 2.5 | 3 | 3.5 | 4 | 5 | 6 | 8 |

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 until 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 triphosphate 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 polymerase is tested for contaminating activities as stated below.

Test buffer: 10mM Tris-HCl; 1.5mM MgCl₂; 50mM KCl; pH 9.0 (20°C). 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°C for 16 hours. The amount of enzyme showing no degradation of the lambda DNA is stated under "Endo".

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)



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