Taq DNA Polymerase

PRODUCT INFORMATION

Component	E00007-1000	E00007-50000	Customized Quotes via email
Taq DNA Polymerase, 5 U//µl	200 µl	50 x 200 µl	Customized
10X Reaction Buffer	2 x 1.5 ml	100 x 1.5 ml	

Description

Taq DNA Polymerase is a thermostable DNA Polymerase isolated from an *E. coli* strain that carries the *Taq* DNA polymerase gene. *Taq* DNA polymerase is the most common polymerase used for PCR reactions

Applications

Taq DNA Polymerase can be used in most applications including the following:

- PCR.
- 3' A-tailing of blunt ends.
- Primer extension.

Source

E. coli strain that carries the *Taq* DNA polymerase gene

Unit Definition

One unit of the *taq* DNA polymerase is defined as the amount of enzyme that can incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage Buffer

The *taq* DNA polymerse is supplied in 5 units/µl in 20 mM Tris HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

10X Reaction Buffer (with Mg^{2+})

500 mM KCl, 100 mM Tris HCl (pH 9.0 at 25°C), 15 mM $MgCl_2$, 1% Triton X-100 Buffer. This buffer is optimized for use with 200 µM dNTPs.

Note: If the reaction is performed without this buffer, then add 0.1% Triton X-100 (final concentration) to ensure high activity

PROTOCOL FOR PCR USING TAQ DNA POLYMERASE

This is a guideline for PCR amplification with taq DNA polymerase from GenScript. This guideline can help you optimize PCR reaction conditions including incubation times, temperatures, and amount of template DNA, all steps may vary and must be individually determined.

Key notes before starting

1. Prepare the reaction mixtures in a separate area that used for DNA preparation or PCR product analysis.
2. Do use disposable tips containing hydrophobic filters to minimize cross contamination.

Things to do before starting

If required, prepare a dNTP mix containing 10 mM of each dNTP. Make sure to store this mix in aliquots at –20°C.

Note: For convenient use , we also provide a dNTP Mix, 10 mM each (Cat. No. C01689), this dNTP mix contains stabilizers and can retain over 50% activity for one month even at 37℃

Procedure

Note: For convenience, to simply save the time of whole procedure, please use a High-Stability PCR Kit (Cat. No. L00342) containing *Taq* DNA polymerase, 10X buffer, and stabilized 10 mM dNTP (Cat. No. C01689), or a single *Taq* PCR Master Mix (Cat. No.E00019) which includes a premixed solution containing *Taq* DNA Polymerase,10X Reaction Taq Buffer and dNTPs (Cat. No. D0056)

1. Thaw 10X Reaction *Taq* Buffer, dNTP mix, and primer solutions at room temperature or on ice

Keep the solutions on ice after complete thawing. **It is important to mix the PCR Master Mix before use to avoid localized differences in salt concentration**

2. Prepare a master mix according to table 1.

The master mix contains all of the components needed for PCR reaction except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should be included in every experiment. The optimal Mg^{2+} concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the 1×Reaction *Taq* Buffer will produce satisfactory results. **Keep the master mix on ice.**

Note: The Mg^{2+} concentration provided by the 10X Reaction *Taq* Buffer (Cat. D0056) will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg^{2+} concentration. *Taq* DNA Polymerase without Mg^{2+} (Cat.No.E00008) includes a single tube of 25 mM Mg^{2+} to adjust the concentration of Mg^{2+}.

Table 1. Reaction Composition Using Taq DNA Polymerase

Component	**Volume/reaction**	**Final concentration**
Master mix	Depend on the rexperiment	Depend on the rexperiment
10X Reaction *Taq* Buffer (Contains 15 mM $MgCl_2$)	5 µl	1X
dNTP mix (10 mM each)	1 µl	200 µM of each dNTP
Primer A	Variable	0.1–2 pM
Primer B	Variable	0.1–2 pM
Template DNA(Simple)	Variable	1-100 ng/reaction
Template DNA(complicated)	Variable	0.1-2 µg/reaction
Distilled water	Variable	----
Taq DNA Polymerase, added at step 4	0.2-0.5 µl	1-2.5 units/reaction
Total volume	50 µl	----
Note: If bigger reaction volumes are used, please add to the amount of each component accordingly.		

3. Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.

Mix gently by pipetting the master mix up and down a few times. **It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.**

4. Add Taq DNA Polymerase to the individual tubes containing the master mix.

5. Program the thermal cycler according to the protocol.

A typical PCR cycling program is outlined in table 2. **For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.**

Table 2. Thermal Cycler Conditions

Procedure	Time	Temp.	Additional comments
Initial denaturation	3-5min	94°C	NA
3-step cycling	----	----	----
Denaturation	0.1–1 min	94°C	NA
Annealing	0.1–1 min	50–68°C	Approximately 5°C below Tm of primers
Extension	0.3-1min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min/kb DNA.
Number of cycles	20–35		----
Final extension	5-7min	72°C	----

6. For a simplified hot start, proceed as described step a. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.

a. Simplified Hot Start

Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler. In many cases, this simplified hot start improves the specificity of the PCR.

Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

7. PCR product was subjected to agarose gel electrophoresis according to Table 3

Table 3. Thermal Cycler Conditions

%TAE (TBE) agarose gel	PCR product (bp)
0.8	~5 kb
1.0	~1.5 kb
1.5	100bp ~3 kb
2.0	100bp~700bp
3.0	~100bp

Note: There is a non-polluting dyes ,6X Loading Buffer with GelRed（Cat.No. M00120） contains DNA loading buffer and fluorescent nucleic acid dye-GelRed, GelRed and EB have virtually the same spectra, you can directly replace EB with GelRed without having to change your existing imaging system and this dye is sensitivity at least 10 times than EB's.

Finally, the following points supplement

1、This protocol is for PCR cycler with a hot lid. Otherwise, mineral oil needs to be added to prevent evaporation.

2、5% DMSO, 1M betaine, or both can be included in PCR reaction to improve the results when a GC-rich template is used.

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