Life Technologies™
Your Molecular & Cell Technology Partner

LT1012ZE

LIFE TECHNOLOGIES TAQ + dNTPs

Concentration: 5U/µl

Contents:

Taq DNA Polymerase	100µl
10xPCR Buffer(Mg^{2+} Plus)	1.25ml
dNTPs(2.5mM each)	1ml
6xLoading Buffer	1ml

Store at -20°C

For research only

In total 4 vials.

Description

Taq 500U + dNTPs is a thermostable recombinant DNA polymerase derived from thermophilic bacterium *Thermus aquaticus*. Its molecular weight is 94 kDa. Taq DNA Polymerase can amplify DNA target up to 5 kb (simple template). The elongation velocity is 0.9~1.2kb/min (70~75°C). It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20mM TrisCl (pH8.0), 100mM KCl, 3.2mM $MgCl_2$ 1mM DTT，0.1% Triton X-100 ,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

10X PCR Buffer with Mg^{2+}

100mM Tris-HCl(PH 8.8), 500mMKCl, 1%Triton-X-100, 16mM $MgCl_2$

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Applications

- PCR amplification of DNA fragments as long as 5 kb.
- DNA labeling.
- DNA sequencing.
- PCR for cloning.

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Taq DNA Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcent- rifuge tube sitting on ice:

1.1 Recommended PCR assay with PCR Buffer (Mg^{2+} plus)

Reagent	Quantity, for 50μl eaction	Final concentration
Sterile deionized water	variable	-
10X PCR Buffer (Mg^{2+} plus)	5μl	1X
dNTPs （2.5mM each）	4μl	0.2 mM each
Primer I	variable	0.4-1μM
Primer II	variable	0.4-1μM
Taq DNA Polymerase（5U/μl）	0.25-0.5 μl	1.25-2.5U/50 μl
Template DNA	variable	10pg-1μg
Total		50μl

1.2 Recommended PCR assay with PCR Buffer (Mg^{2+} free)

Reagent	Quantity, for 50μl of reaction	Final concentration
Sterile deionized water	variable	-
10X PCR Buffer (Mg^{2+} free)	5μl	1X
dNTPs （2.5mM each）	4μl	0.2 mM each
Primer I	variable	0.4-1μM
Primer II	variable	0.4-1μM
25mM Mg^{2+}	variable	1-4mM
Taq DNA Polymerase （5U/μl）	0.25-0.5 μl	1.25-2.5U/50 μl
Template DNA	variable	10pg-1μg
Total		50μl

Table for selection of 25 mM $MgCl_2$ solution volume in 50μl reaction mix :

Final Mg^{2+} conc.	1.0mM	1.5mM	2.0mM	2.5mM	3mM	4mM
Mg^{2+} Stock	2μl	3μl	4μl	5μl	6μl	8μl

Recommendations with Template DNA in a 50μl reaction volume

Human genomic DNA	0.1 μg-1 μg
Plasmid DNA	0.5 ng-5 ng
Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 ng

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 μl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94℃	3 minutes
25-35 Cycles	94℃ 55-68℃ 72℃	30 seconds 30 seconds 1 minutes
Final Extension	72℃	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide stain- ing. Use appropriate molecular weight standards.

Notes on cycling conditions

- Recombinant Taq DNA Polymerase is the enzyme of choice for most PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of Taq DNA Polymerase in PCR is $2.2x10^{-5}$ errors per nt per cycle; the accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is $4.5x10^{-4}$ (determined according to the modified method described in).
- Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Guidelines for preventing contamination of PCR reaction

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucl-eases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 10U-Taq DNA Polymerase with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 10U-Taq DNA Polymerase with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 10U Taq DNA Polymerase with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

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