Taq DNA Polymerase

#Cat: NB-03-0083 Size: 500U

Concentration : 5U/µl

Contents

Taq DNA Polymerase	100 µl
10× PCR Buffer (Mg^{2+} Free)	1.25 ml
6× Loading Buffer	1 ml
25mM $MgCl_2$	1.25 ml

Store at -20°C

For research use only. In total 4 vials.

Description

Taq DNA Polymerase 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 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 Tris-HCl (pH 8.0), 100mM KCl, 3.2mM $MgCl_2$, 1mM DTT, 0.1% Triton X-100, 0.1% Tween20, 0.2mg/ml BSA, 50% (V/V) glycerol.

10× PCR Buffer without Mg^{2+}

100mM Tris-HCl (pH 8.8), 500mM KCl, 1% Triton X-100

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.

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1. **Add the following components to a sterile microcentrifuge tube sitting on ice:**

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

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× PCR Buffer (Mg^{2+} plus)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM 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		50 µl

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

Reagent	Quantity	Final concentration
Sterile deionized water	variable	-
10× PCR Buffer (Mg^{2+} free)	5 µl	1×
dNTPs (10mM each)	1 µl	0.2mM each
Primer I	variable	0.4-1µM
Primer II	variable	0.4-1µM
25mM Mg^{2+}	variable	1.0-4.0mM
Taq DNA Polymerase (5U/µl)	0.25-0.5 µl	1.25-2.5U/50 µl
Template DNA	variable	10pg-1µg
Total		50 µl

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

Final Mg^{2+} Conc. (mM)	1.0	1.5	2.0	2.5	3.0	4.0
Mg^{2+} (25mM)	2 µl	3 µl	4 µl	5 µl	6 µl	8 µl

Recommendation amounts of template DNA in a 50 µl reaction mix:

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

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°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 minutes
Final Extension	72°C	10 minutes

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

5. Analyze the amplification products by agarose gel

6. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleicacid dye staining. 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 2.2×10^{-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 4.5×10^{-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 contaminations 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, exodeoxyribonucleases 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.