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Product Information

Version 6.2, Revision 2013-07-02

Product: **Code:** **Concentration:**	**Taq DNA Polymerase** A1003/A1004 5U/μl
Unit Definition:	One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTPs into an acid-insoluble form in 30 minutes at 70°C using herring sperm DNA as substrate.
Description:	**Taq DNA Polymerase** is a highly thermostable DNA polymerase of the thermophilic bacterium *Thermus aquaticus*. Its molecular weight is 94 kDa. The enzyme catalyzes 5' to 3' synthesis of DNA, has no detectable 3' to 5' exonuclease (proofreading) activity and possesses low 5' to 3' exonuclease activity. In addition, *Taq* DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Biomatik's *Taq* DNA Polymerase is ideal for standard PCR of templates 5 kb or shorter, featuring: • The half life is more than 40 minutes at 95°C. • Generates PCR products with 3'-dA overhangs. • Incorporates modified nucleotides (e.g., biotin-, digoxigenin-, fluorescently-labelled nucleotides). • The error rate in PCR is $2.2x10^{-5}$ per nt per cycle.
Applications:	Routine PCR amplification of DNA fragments **up to 5kb** DNA labeling DNA sequencing PCR cloning
Storage Buffer:	20mM TrisCl (pH8.0), 100mM KCl, 3.2mM $MgCl_2$, 1mM DTT, 0.1% Triton X-100, 0.1% Tween 20, 0.2mg/ml BSA, 50% (v/v) glycerol
10X Buffer: **(Mg^{2+} free)**	120mM Tris-HCl (pH 8.8), 500mM KCl, 1% Triton X-100, 100mM Glycine
10X Buffer: **(Mg^{2+} plus)**	120mM Tris-HCl (PH 8.8), 500mM KCl, 1%Triton-X-100, 16mM $MgCl_2$
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.
Storage:	-20°C

This product is distributed for laboratory research only. Not for human or animal diagnostic and therapeutic use.

Standard 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, Mg2+, and template DNA) vary and need to be optimized. PCR reactions should be assembled in DNA-free environment. DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction analysis, should be performed in separate areas. A control reaction, omitting template DNA, should always be performed to confirm the absence of contamination.

PCR Reaction Setup

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add components, in the following order, into a thin-walled sterile 0.2ml PCR tube sitting on ice. The following control PCR reactions should be performed in parallel with your experiments to ensure that Taq DNA polymerase is working properly.

Recommended PCR with 10X Buffer (Mg2+ free)		
Reagent	Quantity, for 50µl of reaction mixture	Final concentration
Sterile Deionized Water	variable	-
10x PCR Buffer (Mg2+ free)	5µl	1x
dNTPs (10mM each)	1µl	0.2mM each
Primer I, forward	variable	0.4-1µM
Primer II, reverse	variable	0.4-1µM
25mM Mg2+	variable	1-4mM
Taq DNA Polymerase (5U/µl)	0.25-0.5µl	1.25-2.5U/50µl
DNA Template	variable	10pg-1µg
Total		50µl

* We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.

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

Final Mg2+ Concentration	1.0mM	1.5mM	2.0mM	2.5mM	3.0mM	4.0mM
Mg2+ Stock	2µl	3µl	4µl	5µl	6µl	8µl

Recommended PCR with 10X Buffer (Mg2+ plus)		
Reagent	Quantity, for 50µl of reaction mixture	Final concentration
Sterile Deionized Water	variable	-
10x PCR Buffer (Mg2+ free)	5µl	1x
dNTPs (10mM each)	1µl	0.2mM each
Primer I, forward	variable	0.4-1µM
Primer II, reverse	variable	0.4-1µM
25mM Mg2+	variable	1-4mM
Taq DNA Polymerase (5U/µl)	0.25-0.5µl	1.25-2.5U/50µl
DNA Template	variable	10pg-1µg
Total		50µl

* We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.

Recommendations with Template DNA in a 50µl reaction volume

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

3. Gently vortex the sample and briefly centrifuge to collect all drops from walls of the tube.
4. Overlay the sample with 50% of the total reaction volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thermo cycler is equipped with a heated lid.
5. Perform 25-35 cycles of PCR amplification.

Initial Denaturation	94°C	3 mins
25-35 Cycles	94°C 55-68°C 72°C	30 secs 30 secs 1 min
Final Extension	72°C	10 mins

6. Incubate for an additional 10 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C.
7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide. Use appropriate MW markers.

Notes

1. DNA Template: Usually the amount of template DNA is in the range of 0.01-1ng plasmid or phage DNA and 0.1-1µg for genomic DNA, for a total reaction mixture of 50µl.
2. Primers: The PCR primers are usually 15-30 nucleotides in length, longer primers provide higher specificity. The GC content of primer should be 40-60%. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, and the melting temperature of flanking primers should not differ by more than 5°C. If the primer is shorter than 25 nucleotides, the approx. melting temperature is calculated using the formula as: **TM=4(G+C) + 2(A+T)**.
3. $MgCl_2$ concentration: Since Mg2+ ions form complex with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each experiment. In our experiments, at a final dNTP concentration of 200µM, 2mM $MgCl_2$ concentration is suitable in most case.
4. dNTPs: The final concentration of each dNTP in the reaction mixture is usually 200µM.
5. Taq DNA polymerase: Usually 2.5-5U of Taq DNA polymerase is used in the 50µl of reaction mix. Higher Taq DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are presents in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Taq DNA polymerase (5U) may be necessary to obtain a better yield of amplification products.
6. Cycling conditions: Usually denaturation for 0.5-2min at 94-95°C is sufficient; the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex; usually the extending step is performed at 70-75°C. Recommended extending time is 1min for the synthesis of PCR fragments up to 2Kb. When larger DNA fragments are amplified, the extending time is usually increased by 1min for each 1Kb.
7. Number of cycles: 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. For less than 10 copies of template DNA, 40 cycles should be performed. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.
8. Final extending step: After the last cycle, the samples are usually incubated at 72°C for 5-15min to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of Taq DNA polymerase adds extra A nucleotides to the 3'-ends of PCR products.
9. Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
10. 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 most PCR reactions. Low amounts of starting template may require 40 cycles or more.