Taq PCR Kit

NEB #E5000S

200 reactions

Version 3.0_6/18

Table of Contents

Kit Components

The *Taq* PCR Kit contains a sufficient supply of recombinant, highly purified *Taq* DNA Polymerase, PCR-qualified buffer solutions, deoxynucleotides and a broad-range, pre-mixed, ready-to-load DNA marker to perform 200 PCR reactions.

Taq DNA Polymerase with Standard *Taq* Buffer (NEB #M0273)

250 units (5,000 units/ml)

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween® 20, 0.5% IGEPAL® CA-630, and 50% glycerol.

Unit definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol of dNTP into acid insoluble material in 30 minutes at 75°C.

Unit assay conditions: 1X ThermoPol® Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed M13 DNA.

Deoxynucleotide Solution Mix (NEB #N0447)

200 µl (10 mM of each dATP, dCTP, dGTP and dTTP)

Deoxynucleotide Solution Mix is an equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP. These deoxynucleotides are supplied at a 10 mM concentration in Milli-Q water as a sodium salt at pH 7.5.

Standard *Taq* Reaction Buffer (NEB #B9014)

1.5 ml (10X)

This minimal PCR buffer is compatible with most existing PCR platforms and is ideal for use in DHPLC and high throughput applications.

1X Standard *Taq* Reaction Buffer: 10 mM Tris-HCl (pH 8.3 @ 25°C), 50 mM KCl, 1.5 mM $MgCl_2$

Standard *Taq* (Mg-free) Reaction Buffer (NEB #B9015)

1.5 ml (10X)

This version of the Standard *Taq* Reaction Buffer lacks $MgCl_2$ to allow complete control over the final magnesium concentration by adding $MgCl_2$ from the supplied stock solution.

Magnesium Chloride ($MgCl_2$) Solution (NEB #B9021)

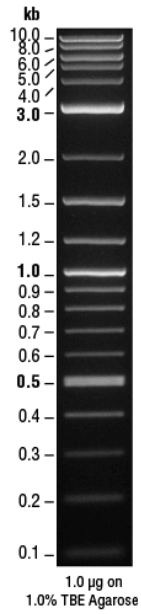
1.5 ml (25 mM)

A 25 mM solution of $MgCl_2$ is provided to allow adjustments to magnesium concentration.

Quick-Load® Purple 1 kb Plus DNA Ladder (NEB #N0550)

200 µl (100 µg/ml)

The Quick-Load Purple 1 kb Plus DNA Ladder is a pre-mixed, ready-to-load molecular weight marker with fragments ranging from 0.1–10 kb. The 0.5, 1.0 and 3.0 kb bands are present at higher concentrations for unambiguous marker identification. The ladder is provided in sample buffer at 100 µg/ml, and 10 µl (1.0 µg) per lane should be used for agarose gel electrophoresis.



Fragment	Base Pairs	DNA Mass
1	10,002	40 ng
2	8,001	40 ng
3	6,001	48 ng
4	5,001	40 ng
5	4,001	32 ng
6	**3,001**	**120 ng**
7	2,017	40 ng
8	1,517	57 ng
9	1,200	45 ng
10	**1,000**	**122 ng**
11	900	34 ng
12	800	31 ng
13	700	27 ng
14	600	23 ng
15a	**517**	**124 ng**
15b	**500**	
16	400	49 ng
17	300	37 ng
18	200	32 ng
19	100	61 ng

Introduction to PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (1). PCR amplifies specific DNA sequences exponentially by using multiple cycles of a three-step process. First, the double-stranded DNA template is denatured at a high temperature. Sequence-specific primers are then annealed to sites flanking the target sequence. A thermostable DNA polymerase, such as *Taq* DNA Polymerase (2–6), then extends the annealed primers, thereby doubling the amount of the original DNA sequence. This newly synthesized product then becomes an additional template for subsequent cycles of amplification. These three steps are repeated for 20 to 30 cycles, resulting in a 10^5–10^9 fold increase in target DNA concentration.

Protocol for a Routine PCR Reaction

All components should be mixed and spun down prior to pipetting. These recommendations serve as a starting point; in order to maximize amplification the reaction conditions may require optimization (see page 4).

1. Prepare the following 50 µl reaction in a 0.5 ml PCR tube on ice:

COMPONENT	VOLUME (µl)	FINAL CONCENTRATION
Standard *Taq* Reaction Buffer (10x)	5 µl	1X
Deoxynucleotide Solution Mix (10 mM)	1 µl	200 µM
Upstream Primer (10 µM stock)	1 µl	0.2 µM (0.05-1.0 µM)
Downstream Primer (10 µM stock)	1 µl	0.2 µM (0.05-1.0 µM)
DNA Template	Determined by user	1 pg-1 ng plasmid DNA 1 ng- 1 µg genomic DNA
Taq DNA Polymerase*	0.25 µl	1.25 units/50 µl PCR
Nuclease free water	Bring reaction to a final volume of 50 µl	

**Due to the difficulties in pipetting small volumes of enzyme, Taq DNA Polymerase can be diluted in Diluent F (NEB #B8006) or 1X reaction buffer. For example, 1 µl of Taq DNA Polymerase is mixed with 3 µl of diluent and 1 µl of that mixture is added to the reaction. Enzyme diluted in Diluent F can be stored at –20°C for future use.*

2. Gently mix the reaction and spin down in microcentrifuge:

If the thermocycler does not have heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation

3. Cycling Conditions for a Routine PCR Reaction:

CYCLE STEP	TEMP	TIME	CYCLES
Initial Denaturation	95°C	30 seconds	1
Denaturation Annealing Extension	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute per kb	30
Final Extension	72°C	5 minutes	1
Hold	4°C	∞	

PCR Optimization

Taq DNA Polymerase is an enzyme widely used in PCR (7). The following guidelines are provided to ensure successful PCR using New England Biolabs' *Taq* DNA Polymerase. These guidelines cover routine PCR reactions. PCR of templates with high GC content, high secondary structure, low template concentrations, or amplicons greater than 5 kb may require further optimization.

1. **DNA Template:** Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. It is also critical that contamination from outside sources, especially previous PCR reactions, be avoided.

   Approximately 10^4 copies of the target DNA are required to detect a product in 25–30 cycles of PCR. Recommended amounts of template DNA for a 50 µl reaction are 1 pg–1 ng of plasmid or viral templates and 1 ng–1 µg of genomic templates. In general, the higher the DNA concentration and number of cycles, the lower the primer specificity. High DNA concentrations can be advantageous when fewer cycles are desired to increase the fidelity of the reaction.

2. **Primers:** Oligonucleotide primers are generally 20–30 nucleotides in length and ideally have a GC content of 40–60%, with GC residues spaced evenly within the primer. Calculated melting temperatures (T_m) for the two primers should be above 45°C and the T_m for the two primers should be within 5°C of each other.

   The final concentration of each primer in a PCR reaction may be 0.05–1.0 µM (typically 0.1–0.5 µM). Higher concentrations increase the possibility of secondary priming, which potentially creates spurious amplification products.

3. **Magnesium Concentration:** A magnesium concentration of 1.5–2.0 mM is optimal for most PCR products generated with Taq DNA Polymerase. Optimization normally involves supplementing the magnesium concentration in 0.5 or 1.0 mM increments.

4. **Deoxynucleotides:** The final concentration of dNTPs is typically 200 µM of each deoxynucleotide. Concentrations > 500 µM should be avoided.

5. ***Taq* DNA Polymerase Concentration:** Taq DNA Polymerase is normally present at a final concentration of 25 units/ml (1.25 units/50 µl reaction), but can range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications. Enzyme dilutions can be made in 1X reaction buffer if used immediately. If dilutions must be stored, use Diluent F (NEB #B8006).

6. **Hot Start:** Non-specific primed synthesis during the assembly of the reaction prior to PCR cycling has been identified as a source of nonspecific products in some PCR reactions. These undesired products can often be avoided by assembling all components on ice, adding the polymerase last and immediately transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C). If this approach continues to yield non-specific products, use of a hot start polymerase is recommended. OneTaq® Hot Start DNA Polymerase (NEB #M0481) is available from NEB to accommodate these situations.

7. **DNA Contamination:** To minimize DNA contamination during reaction setup, it is recommended that positive-displacement pipets or aerosol barrier tips be used to aliquot and mix reagents. In order to further minimize contamination, as well as decrease pipetting errors, users are encouraged to make master mixes of reagents.

8. **Master Mix:** When setting up multiple reactions it is faster and more accurate to create a master mix of the components that are common to all reactions. In general, this involves creating a stock solution of polymerase, deoxynucleotides, reaction buffer, water, and occasionally primers. The master mix is then aliquotted and mixed with the DNA template and any required primers.

9. **Denaturation Temperature and Duration:** An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 95°C is recommended.

During thermocycling, a 15–30 second denaturation at 95°C is recommended, although this can depend on the thermocycler and tubes used. Consult the product literature accompanying the thermocycler being used for more specific recommendations.

10. **Annealing Temperature and Duration:** Annealing temperatures should be chosen to match the T_m values of the primer pair and are typically 45–68°C. If extra bands are observed, higher annealing temperatures should be considered. The absence of product can indicate the need for a lower annealing temperature. Annealing times of 15–60 seconds are usually adequate. We recommend using NEB's T_m Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

11. **Extension Time:** Extensions are normally done at 68°C. As a general rule, extension times of one minute per kb should be used. For products less than one kb, an extension time of 45–60 seconds should be used. A final extension of 5 minutes at 68°C is recommended.

Troubleshooting

OBSERVATION	POSSIBLE CAUSE(S)	SOLUTION(S)
No amplification product	Poor primer design	• Check the integrity of the RNA by denaturing agarose gel electrophoresis (3). • Increase length of primer.
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence.
	Insufficient primer concentration	• Increase primer concentration to 0.1-0.5 µM.
	Missing reaction component	• Repeat reaction setup.
	Target sequence not present in template DNA	• Try other sources of template DNA.
	Poor reaction conditions	• Optimize (Mg^{++}), annealing temperature and extension time. • Thoroughly mix Mg^{++} solution. • Check primer concentrations.
	Questionable template quality	• Analyze DNA via gel electrophoresis after incubation with Mg^{++}
	Inhibitory substance in reaction	• Decrease sample volume. • Try alcohol precipitation or drop dialysis to further purify DNA.
	Insufficient number of cycles	• Rerun the reaction with more cycles.
	Incorrect thermocycler programming	• Check program, verify times and temperatures.
	Inconsistent block temperature	• Test calibration of heating block.
	Reaction tubes or solutions contaminated	• Autoclave tubes prior to use to eliminate biological inhibitors.

OBSERVATION	POSSIBLE CAUSE(S)	SOLUTION(S)
Multiple or non-specific products	Premature *Taq* DNA Polymerase replication	• Set up reactions on ice with chilled components. Add samples to pre-heated (95°C) thermocycler. • Try a hot start polymerase, such as One*Taq* Hot Start DNA Polymerase (NEB#M0481).
	Primer annealing temperature too low	• Raise annealing temperature in 2°C increments.
	Insufficient mixing of reaction buffer	• Reaction buffer must be thoroughly mixed.
	Improper Mg^{++} concentration	• Adjust Mg^{++} concentration in 0.5 mM increments.
	Poor primer design	• Verify that primers have no complementary regions- either internally or to each other. • Try longer primers. • Avoid GC-rich 3' ends.
	Excess primer	• Reduce primer concentration to 0.1-0.5 µM.
	Contamination with exogenous DNA	• Analyze DNA via gel electrophoresis after incubation with Mg^{++}
	Multiple target sequences in template DNA	• Decrease sample volume. • Try alcohol precipitation or drop dialysis to further purify DNA.

References

1. Liao, J. and Gong, Z. (1997) *Biotechniques,* 23, 368–370.
2. Van Gilst, M.R. et al. (2005) *PLoS Biology*, 3, 301–312.
3. Sambrook, J. and Russel, D.W. (2001) Molecular Cloning: A Laboratory Manual (3rd Ed.) Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
4. Don, R.H. et al. (1991) *Nucleic Acid Research,* 19, 4008.
5. Aguila et al. (2005) *BMC Molecular Biology,* 6, 9.

Quality Controls

5 kb Lambda PCR

25 cycles of PCR amplification of 5 ng Lambda DNA with 1.25 units of *Taq* DNA Polymerase in the presence of 200 µM dNTPs and 0.2 µM primers in Standard *Taq* Reaction Buffer results in the expected 5 kb product.

3´– 5´ Exonuclease Activity

Incubation of a 20 µl reaction in ThermoPol™ Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´- 5´ degradation as determined by capillary electrophoresis.

Endonuclease Activity

Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Reaction Buffers

The supplied NEB reaction buffers and supplements are free of detectable nucleases.

Deoxynucleotide Solution

Deoxynucleotide solutions are certified free of detectable nucleases and phosphatases.

Ordering Information

NEB #	PRODUCT	SIZE
E5000S	*Taq* PCR Kit	200 reactions
KIT COMPONENTS SOLD SEPARATELY		
M0273S/L/X	*Taq* DNA Polymerase with Standard *Taq* Buffer	400/2,000/4,000 units
B9014S	Standard *Taq* Reaction Buffer Pack	6 ml
B9015S	Standard *Taq* (Mg-free) Reaction Buffer Pack	6 ml
B9021S	Magnesium Chloride ($MgCl_2$) Solution	6 ml
N0550S/L	Quick-Load Purple 1 kb Plus DNA Ladder	250/750 gel lanes
N0447S/L	Deoxynucleotide (dNTP) Solution Mix	8/40 µmol of each
COMPANION PRODUCTS		
M0481S/L/X	One*Taq* Hot Start DNA Polymerase	200/1,000/12,000 units
M0267S/L/X	*Taq* DNA Polymerase with ThermoPol Buffer	400/2,000/4,000 units
M0320S/L	*Taq* DNA Poly w/Standard *Taq* (Mg-free) Buffer	400/2,000 units
B9004S	ThermoPol Reaction Buffer Pack	6 ml
N0446S	Deoxynucleotide (dNTP) Solution Set	25 µmol of each

www.neb.com

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com