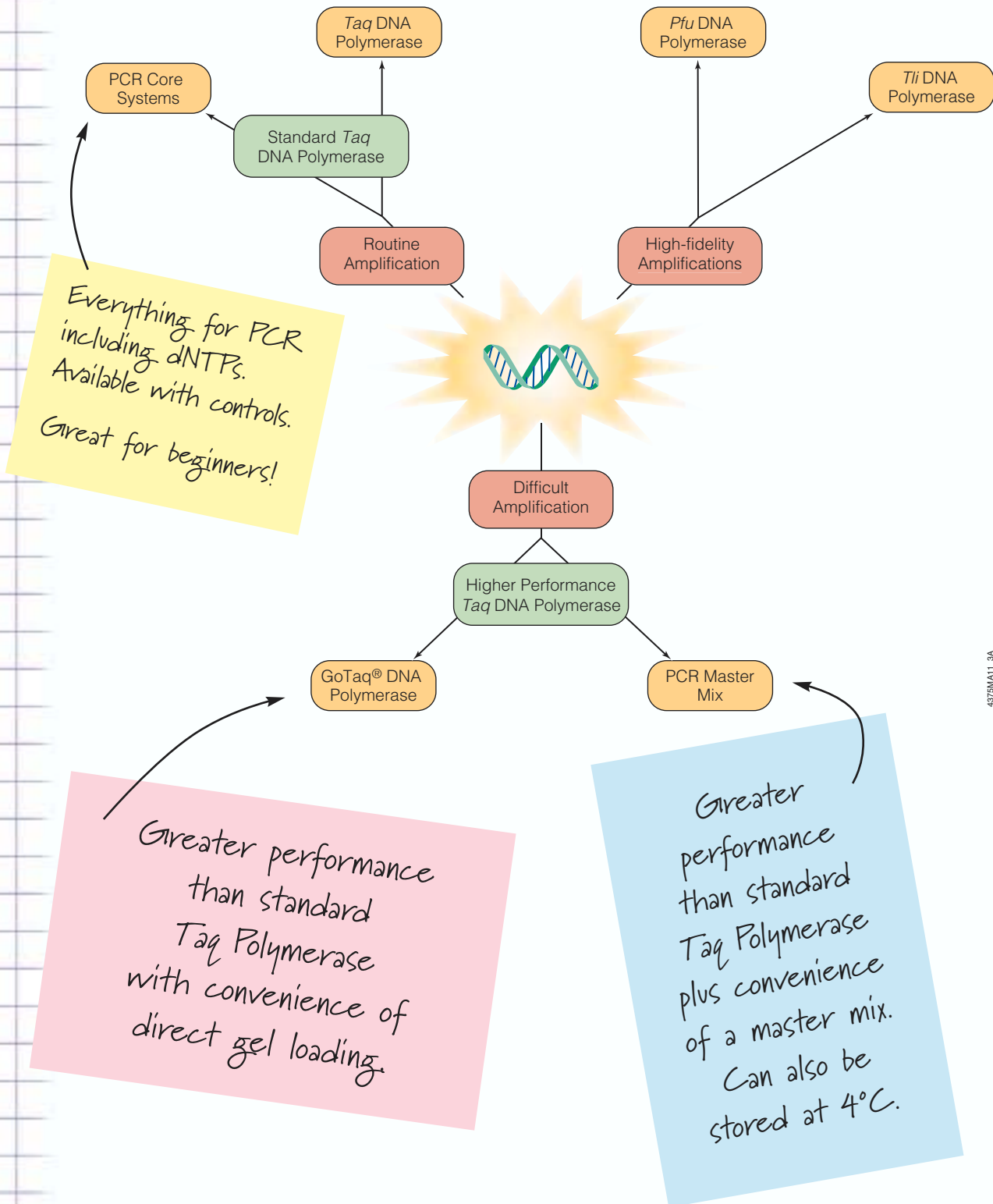


Amplifying DNA

Promega offers several options for routine, difficult and high-fidelity PCR.



4375MA11_3A

Amplifying DNA

Overview

Denature... Anneal... Extend... PCR amplification led to a revolution in molecular biology in the 1980's. PCR is a relatively simple technique by which a DNA or cDNA template is amplified many thousand- or millionfold quickly and reliably, generating sufficient material for subsequent analyses.

The PCR process is exquisitely sensitive. While most biochemical analyses—including nucleic acid detection with radioisotopes—require the input of significant amounts of biological material, the PCR process requires very little starting material. This feature makes the technique extremely useful, not only in basic research, but also for applications such as genetic identity testing, forensic analysis, industrial quality control and in vitro diagnostics.

The availability of such a powerful tool has led to significant developments in answering biological questions. Many adaptations of the original PCR method have been published, and numerous factors that are critical for accurate amplification have been identified.

How Much Enzyme is Needed in a Reaction?

Promega recommends using 1.25 units of thermostable DNA polymerase per 50 μ l amplification reaction. For most applications, the enzyme will be in excess. The inclusion of more enzyme will not significantly increase yield. Increased amounts of enzyme and excessively long extension times will increase the likelihood of artifacts due to the intrinsic 5'→3' exonuclease activity of *Taq* DNA Polymerase^(e) and other non-proofreading DNA polymerases. Artifacts are generally seen as smeared bands in ethidium bromide-stained agarose gels.

The most frequent cause of excessive enzyme levels is pipetting error. Accurate dispensing of submicroliter (<1 μ l) volumes of enzyme solutions in 50% glycerol is nearly impossible. We strongly recommend making reaction master mixes sufficient for the number of reactions being performed. A master mix increases the volume of pipetted reagents and reduces pipetting errors.

For more information on reaction optimization, see pp. 16–19.

Typical Reaction with *Taq* DNA Polymerase.

Nuclease-Free Water	to 50 μ l final
Reaction Buffer(10X or 5X)	1X
dNTPs	0.2mM each
<i>Taq</i> DNA Polymerase	1.25u
MgCl ₂ *	0.5–4.0mM
Downstream primer	1 μ M (50pmol)
Upstream primer	1 μ M (50pmol)
Template	10 ⁴ copies

*Some reaction buffers contain Mg²⁺, and additional MgCl₂ may not be required. The optimal Mg²⁺ concentration depends on the template but is typically in the range 0.5–4mM.

Assemble reactions on ice in the order listed. Be sure to vortex the MgCl₂ solution, primers, dNTPs and Reaction Buffer prior to addition. When using a thermal cycler without a heated lid, overlay the reaction with 1–2 drops of mineral oil to prevent evaporation.

Setting up reactions with a proofreading polymerase?

Proofreading enzymes like to digest free primers due to their 3'→5' exonuclease activity. Always assemble the reaction on ice and add the proofreading polymerase last, just prior to placing the tubes in a preheated 94–95°C thermal cycler. See p. 23 for more information.

Vortex all MgCl₂-containing solutions thoroughly prior to use! MgCl₂ solutions can form a concentration gradient upon thawing. Failure to vortex is a common source of PCR failure!

Amplifying DNA

Example Cycling Conditions for *Taq* DNA Polymerase.

Step	Temperature	Time (minutes)	Cycles
Initial Denaturation ^(a)	95°C	2	1
Denaturation	95°C	0.5–1	} 25–35
Annealing	42–65°C ^(b)	0.5–1	
Extension	72°C	1 min/kb ^(b,c)	
Final Extension	72°C	5	1
Soak	4°C	indefinite	1

^(a) Reactions are placed in a thermal cycler that has been preheated to 95°C. The thermal cycling protocol has an initial denaturation step where samples are heated at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Initial denaturation of longer than 2 minutes at 95°C is usually unnecessary and may reduce yield. (Some hot start polymerases require pre-incubation at 95°C to activate the polymerase prior to the 2-minute denaturation step.)

^(b) Annealing temperature should be optimized for each primer set based on the primer melting temperature (T_m). See section on primer design (p. 16).

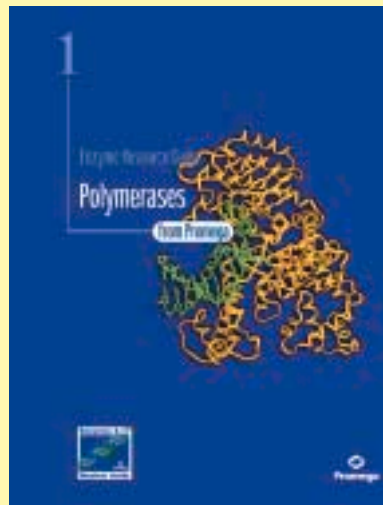
^(c) The extension time should be at least 1 minute/kilobase of target. Typically, anything smaller than 1 kb uses a 1-minute extension, 2 minutes for >1kb, 3 minutes for >2kb, 4 minutes for >3kb, etc.

Using a Proofreading Polymerase?

Proofreading polymerases work a little slower than non-proofreading polymerases. Be sure to increase the extension time to at least 2 minutes per kilobase. Also, you may need 2–3 more cycles.

Want to explore the enzymology of Thermostable DNA Polymerases more thoroughly?

Go to our online Polymerase Guide at:
www.promega.com/guides/



4481CA

Comparison of Properties for Some Commonly-Used Thermostable DNA Polymerases.

Characteristic	Thermostable DNA Polymerase						
	<i>Taq</i> / Ampli <i>Taq</i> ®	Ampli <i>Taq</i> Gold® Platinum® <i>Taq</i>	<i>Tfi</i>	<i>Tth</i>	Vent® (<i>Tli</i>)	Deep Vent®	<i>Pfu</i>
Resulting DNA ends	3' A	3' A	3' A	3' A	>95% Blunt	>95% Blunt	Blunt
5'→3' exonuclease activity	Yes	Yes	Yes	Yes	No	No	No
3'→5' exonuclease activity	No	No	No	No	Yes	Yes	Yes

Amplifying DNA

Optimization of PCR

Magnesium Concentration

Magnesium concentration is an important factor to optimize when performing PCR. The optimal Mg^{2+} concentration varies depending on the primers, template, DNA polymerase, dNTP concentration and other factors. *Taq* DNA polymerase is the most common polymerase used for PCR. *Taq* has an optimal Mg^{2+} range of 1–4mM $MgCl_2$. Other polymerases may have different optimal ranges. For example, *Tth* DNA polymerase has a narrower optimal range (1.5–2.5mM $MgCl_2$), *Tli* DNA Polymerase displays optimal activity at 2–6mM $MgCl_2$, and *Pfu* DNA Polymerase has an optimal range of 2–6mM $MgSO_4$. *Tth* DNA Polymerase has an optimal range of 1–4mM Mg^{2+} but performs better with $MgSO_4$ than with $MgCl_2$.

When using a pair of PCR primers for the first time, it is advisable to perform a magnesium titration in 0.5 or 1.0mM increments to determine the optimal Mg^{2+} concentration. Some primers will amplify equally well at a number of Mg^{2+} concentrations, while others may have very specific Mg^{2+} concentration requirements.

With too little Mg^{2+} , the polymerase will have poor activity. With too much Mg^{2+} , nonspecific amplification can become a problem. Nonspecific PCR products can appear as a smear on a gel or as distinct bands of inappropriate size. Too much Mg^{2+} can also reduce the fidelity of the DNA polymerase and lead to a higher error rate.

Taq DNA polymerase is commonly supplied with buffers containing a fixed concentration of Mg^{2+} (giving a final concentration of 1.5mM in the final reaction). Most *Taq* DNA polymerase amplifications work well at this Mg^{2+} concentration, and the reaction can still be optimized by adding more Mg^{2+} . *Pfu* DNA polymerase does not have as great a dependence upon Mg^{2+} and is most often supplied with a buffer containing a final concentration of 2mM Mg^{2+} . However, this does not mean that optimization is unnecessary, and the final concentration of Mg^{2+} can be adjusted up to 6mM as needed.

Vortex all $MgCl_2$ -containing solutions thoroughly prior to use!

$MgCl_2$ solutions can form a concentration gradient upon thawing. Failure to vortex is a common source of PCR failure!

Primer Design

PCR primers generally range from 15–30 bases long and are designed to flank the DNA region of interest. Primers should have 40–60% GC content, and care should be taken to avoid sequences that might produce intermolecular or intramolecular secondary structure. To avoid the production of primer-dimers, the 3'-ends of the primers should not be complementary. Primer-dimers unnecessarily sequester primers away from the reaction and result in an unwanted polymerase reaction that competes with the desired PCR product. Avoid three G or C nucleotides in a row near the 3'-end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable products. Ideally, both primers should have nearly identical melting temperatures (T_m), allowing both primers to anneal roughly at the same temperature. The annealing temperature of the reaction is dependent upon the primer with the lowest melting temperature. For assistance with calculating the T_m of any primer, a T_m calculator is provided on the Promega web site at: www.promega.com/biomath

Need to calculate your T_m ?
Go to Promega's BioMath page at:
www.promega.com/biomath

The program returns results from three different published methods for T_m calculation. You can also select Promega primers to examine their T_m and determine the T_m of your own primers in different reaction buffers.

Amplifying DNA

Optimization of PCR (continued)

Annealing Temperature

Annealing temperature is another factor that may need to be optimized in PCR. The melting temperature (T_m) of the PCR primers should be in the range 42–65°C, unless the primers fall into a special class, such as degenerate primers, which have lower T_m . Typically the optimal annealing temperature is $\pm 5^\circ\text{C}$ of the primer with the lowest T_m . Ideally the T_m of both primers will be similar so that the optimal annealing temperatures are close. If the melting temperatures are more than a few degrees apart, one primer may need to be redesigned so that the T_m is closer to that of the other primer. A good starting point is to set the annealing temperature equal to the T_m of the primers. If nonspecific amplification occurs, this is a good indication that the annealing temperature needs to be raised a few degrees. If the PCR reaction yields no product, this may indicate that the annealing temperature is too high and should be reduced by several degrees.

Template Quantity

The amount of template required for successful amplification is dependent upon the complexity of the DNA sample. For example, in a 4kb plasmid containing a 1kb insert, 25% of the input DNA is the target of interest. Conversely, a 1kb gene in the human genome ($3.3 \times 10^9\text{bp}$) represents approximately 0.00003% of the input DNA. Approximately 1,000,000-fold more human genomic DNA is required to maintain the same number of target copies per reaction. Two common mistakes are the use of too much plasmid DNA or too little genomic DNA. If possible, start with up to 10^4 copies of the target sequence to obtain a signal in 25–30 cycles, but do not exceed 10ng/ μl (i.e., 500ng/50 μl reaction).

Reaction annealing temperature should be $\pm 5^\circ\text{C}$ of the T_m of the PCR primer that has the lowest T_m .

Template Quality

The purity and integrity of the DNA template can also be critical. Obviously there are numerous inhibitors that can interfere with amplification. These may be copurified from the original source of the nucleic acid (e.g., the tissue from which the DNA was isolated). Contaminants can also be introduced during the purification process. Examples of common contaminants that can inhibit PCR are phenol, ethanol, as little as 0.01% SDS or other detergents, heparin and salts. These contaminants can usually be removed by a simple phenol:chloroform extraction followed by ethanol precipitation, or by use of a PCR clean-up system (see Chapter 3). Some sample types, such as blood, soil, fungus, plants with high phenolic content, and fecal samples, are problematic because they contain strong PCR inhibitors that can be copurified with the DNA. An easy way to identify inhibitors in your template nucleic acid is to add an aliquot of template to the positive control reaction. If this “spiked” control reaction fails, the template needs to be further purified before amplification.

Test template quality by adding a control template that you know amplifies easily and reliably. Combine your problematic template with 100–1,000 copies of the control template, and amplify the control template. Perform the same amplification with the control template alone. If amplification of the control template fails only when the problematic template is present, inhibitors in the problematic template may be to blame.

How Many Molecules in Your DNA Template?

1 μg of 1kb dsDNA =
 9.12×10^{11} molecules

1 μg of pGEM[®] Vector DNA =
 2.85×10^{11} molecules

1 μg of lambda DNA =
 1.9×10^{10} molecules

1 μg of E. coli genomic DNA =
 2×10^8 molecules

1 μg of human genomic DNA =
 3.04×10^5 molecules

Amplifying DNA

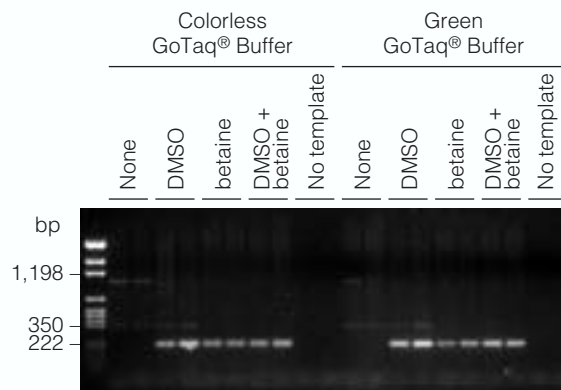
Optimization of PCR (continued)

PCR Enhancers

In some cases it may be helpful to add certain enhancing agents to a PCR, despite all other attempts to optimize conditions. Two good examples are the amplification of GC-rich templates and amplification of templates that form strong secondary structures, which can cause DNA polymerases to stall. GC-rich templates can be problematic due to inefficient separation of the two DNA strands or because of the tendency of GC-rich primers to form intermolecular and intramolecular secondary structures that compete with template annealing. There are many PCR-enhancing agents that act through a number of different mechanisms. PCR-enhancing reagents will not work with all reactions; the beneficial effects are often template- and primer-specific.

Betaine, DMSO and formamide can be helpful when amplifying GC-rich templates. Betaine reduces the amount of energy required to separate the strands of a GC-rich DNA template (1). Dimethylsulfoxide (DMSO) and formamide are thought to aid in the amplification of GC-rich templates in a similar manner by interfering with the formation of hydrogen bonds between the two strands of DNA (2). Some reactions that amplify poorly in the absence of enhancers will give a strong PCR product when betaine (1M), DMSO (1–10%), or formamide (1–5%) are added to the reaction. DMSO concentrations greater than 10%, and formamide concentrations greater than 5% will cause inhibition of *Taq* DNA polymerase and, presumably, other DNA polymerases as well (3).

In some cases, general stabilizing agents such as BSA (0.1mg/ml), gelatin (0.1–1.0%), and nonionic detergents (0–0.5%) can overcome failure to amplify a region of DNA. These additives can increase the stability of the DNA polymerase and may also coat the sides of the PCR tubes so that reagents are not lost through adsorption to the tube walls. BSA has also been shown to overcome the inhibitory effects of melanin on RT-PCR (4). Nonionic detergents, such as Tween®-20, NP-40, and Triton® X-100, have the additional benefit of being able to overcome the inhibitory effects of trace amounts of strong ionic detergents, such as 0.01% SDS (5).



Amplification of a fragment of the human retinoblastoma gene using GoTaq DNA Polymerase with Colorless GoTaq Reaction Buffer or Green GoTaq Reaction Buffer with and without the addition of enhancing agents DMSO and betaine. Amplification reactions contained 500ng human genomic DNA, 0.8µM of each primer and 1.25u GoTaq DNA Polymerase in a final volume of 50µl. Reactions contained no additives, 5% DMSO, 1M betaine or 5% DMSO + 1M betaine as indicated. No-template control reactions were included. Amplification primers and cycling conditions are as published in Frackman, S. *et al.* (1998) Betaine and DMSO: Enhancing agents for PCR. *Promega Notes* **65**, 27–29.

Ammonium ions can make a PCR reaction more tolerant of nonoptimal conditions. For this reason, some PCR reagents include 10–20mM $(\text{NH}_4)_2\text{SO}_4$. Other PCR enhancers include glycerol (5–20%), polyethylene glycol (5–15%), and tetramethyl ammonium chloride (TMAC; 60mM). The effects of these enhancers are very template- and primer-specific. It may be easier to design new primers and determine the optimal conditions for the new primer pair than to do multiple experiments with some of these less useful enhancers.

References

1. Rees, W., Yager, T.D., Korte, J. and von Hippel, P.H. (1993) Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry* **32**, 137–44.
2. Geiduschek, E.P. and Herskovitz, T.T. (1961) Nonaqueous solutions of DNA. Reversible and irreversible denaturation in methanol. *Arch. Biochem. Biophys.* **95**, 114–29.
3. Varadaraj, K. and Skinner, D. (1994) Denaturants or cosolvents improve the specificity of PCR amplification of a GC-rich DNA using genetically engineered DNA polymerases. *Gene* **140**, 1–5.
4. Giambernardi, T.A., Rodeck, U. and Klebe, R.J. (1998) Bovine serum albumin reverses inhibition of RT-PCR by melanin. *BioTechniques* **25**, 564–66.
5. Gelfand, D.H. and White, T.J. (1990) Thermostable DNA polymerase. In: *PCR Protocols: A Guide to Methods and Applications*. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and T.J. White (eds.) Academic Press, San Diego, CA. pp. 129–41.

Amplifying DNA

Optimization of PCR (continued)

Troubleshooting

Most troubleshooting of PCR involves evaluating the possible areas of optimization. Promega has an extensive PCR troubleshooting guide included in the *PCR Core Systems Technical Bulletin #TB254*.

Need a guide to general PCR optimization and troubleshooting?
Get PCR Core Systems Technical Bulletin #TB254 online at:

www.promega.com/tbs/tb254/tb254.html
or request a printed copy from your local Promega representative.

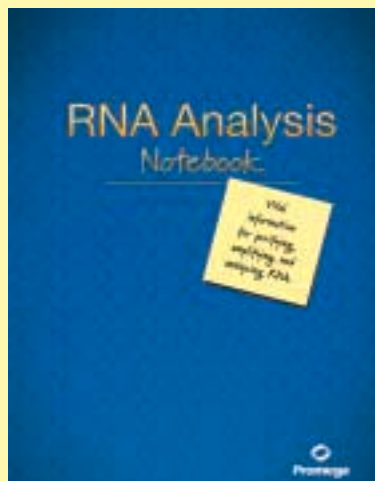
The image shows the cover of the 'PCR Core Systems' technical bulletin. It features the Promega logo in the top right corner. The title 'PCR Core Systems' is prominently displayed at the top. Below the title, there is a small box with the text 'INSTRUCTIONS FOR USE OF PCR CORE SYSTEMS PLEASE ORDER REFERENCE BOOK'. The main body of the cover is a table of contents listing various sections and their corresponding page numbers. The sections include: I. Description (1), II. Product Components (2), III. PCR Protocol (4) with sub-sections A. Protocol (4) and B. Analysis (6), IV. Optimization of PCR (5) with sub-sections A. Reaction PCR (5), B. Magnesium Concentration (6), C. Buffer Concentrations (7), D. Primer Choice (7), E. Enzyme Concentration (8), F. Primer Design (8), G. Template Concentrations (9), H. Primer Annealing Temperature (10), I. Extension Temperature (10), J. Hot Start PCR (11), and K. Reaction Seal Cover Concentration (11), V. Using Controls in PCR (11), VI. Troubleshooting PCR (12), VII. References (14), VIII. Appendix (16) with sub-sections A. Composition of Buffers and Solutions (16), B. Related Products (16), and C. Supplementary Bibliography (17), and IX. Experienced User's Remarks (18). At the bottom, there is a 'Description' section starting with 'The PCR Core Systems I and III are designed for the exponential amplification of specific regions of DNA using the polymerase chain reaction (PCR) with the Taq DNA Polymerase II, a thermostable enzyme isolated from Thermus aquaticus. PCR Core System I provides the reagents and buffers necessary for amplification. PCR Core System II provides the same reagents as System I but also includes protein cofactors and enhancers. Please see Section V to a discussion of applications in PCR. Although PCR is simple in theory, it can benefit from optimization of several parameters. This technical bulletin provides important and convenient information about optimizing PCR and includes protocols and troubleshooting tips for successful PCR amplification.'

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Scientists serving
scientists.

RT-PCR

Looking for information, tips and techniques for RT-PCR? Request the free *RNA Analysis Notebook*. Ask for literature #BR120 from your local Promega distributor or Promega Representative. Also available online at: www.promega.com/guides/



Amplifying DNA

Routine PCR

PCR Master Mix: Robust, Convenient Amplification

Promega's PCR Master Mix^(f,g) is designed for the rapid and convenient amplification of many common genomic and cDNA templates (1). PCR Master Mix, formulated as a 2X solution, offers a single-tube format for PCR setup, reducing pipetting times, steps and errors as well as greatly reducing reagent waste. All necessary PCR components, except for primers and template DNA, are contained in the Master Mix. Stability is also a key feature of PCR Master Mix, which can be stored for up to 24 months at 4°C or be put through as many as 20 freeze-thaw cycles without loss of performance. PCR Master Mix provides 1.25u of *Taq* DNA Polymerase, Reaction Buffer, 200µM of each dNTP, and 1.5mM Mg²⁺ in the final reaction.

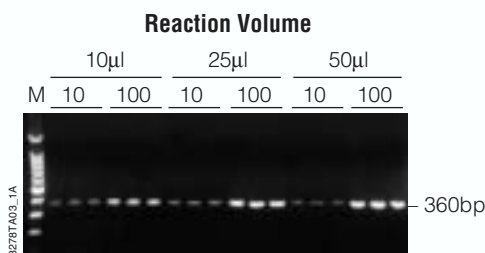
Reference

- Denhart, M. and Doraiswamy, V. (2001) Performance advantages designed into Promega's PCR Master Mix. *Promega Notes* **78**, 9–12.

Typical Reaction Set-Up with PCR Master Mix.

Template (up to 10 ⁴ copies of target)	Xµl
Primers (50pmol each or 1µM final conc.)	Yµl
Nuclease-Free Water (provided)	Zµl
PCR Master Mix*	25µl
Total Volume	50µl

* Provides dNTPs (200µM each), Mg²⁺ (1.5mM), and *Taq* DNA Polymerase (1.25u) at the final 1X concentration.



Scalability of PCR Master Mix. The 360bp α 1-antitrypsin message was amplified from 10 or 100 copies of Human Genomic DNA (Cat.# G3041) in 10, 25 and 50µl reaction volumes as indicated.

PCR Master Mix

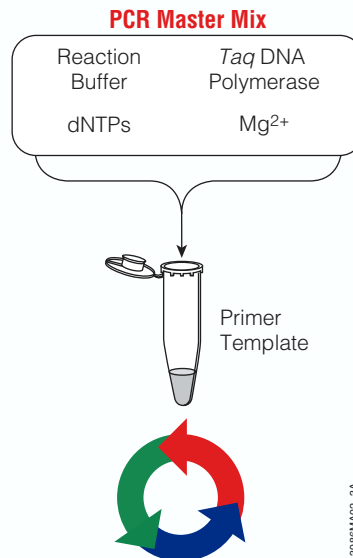
Cat. #: M7501 (10 reactions)
M7502 (100 reactions)
M7505 (1,000 reactions)

Protocol:

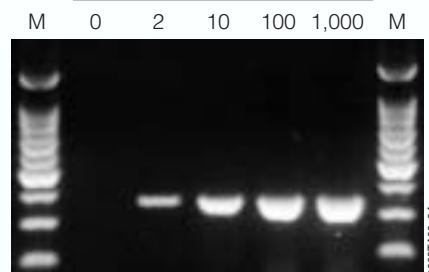
www.promega.com/tbs/9pim750/9pim750.html

Citations detailing use of PCR Master Mix online at:

www.promega.com/citations/



Template Copies per Reaction



Detection of low copy number templates using PCR Master Mix. A 360bp portion of the single-copy α 1-antitrypsin gene was amplified from the indicated amounts of Human Genomic DNA (Cat.# G3041). Lane M, 100bp DNA Ladder (Cat.# G2101).



PCR Master Mix in two-step RT-PCR. Amplification of a 533bp portion of the caspase-3 cDNA from 20µl reverse transcription reaction. Reverse transcription was performed using the ImProm-II™ Reverse Transcription System (Cat.# A3800) and the indicated amount of total RNA. The cDNA in the entire 20µl reaction was amplified by adding 15µl of PCR Master Mix, 2µl of gene-specific primers and 13µl of Nuclease-Free Water (Cat.# P1195). Further details of the experiment may be found in Miller, K., Moravec, R. and Riss, T. (2001) An integrated approach to studying apoptosis: From gene expression to cellular events. *Cell Notes* **2**, 4–6.

Cell Notes and Promega Notes are available online at: www.promega.com or upon request.

Amplifying DNA

Routine PCR (continued)

GoTaq® DNA Polymerase: Direct-to-Gel Amplification

GoTaq DNA Polymerase^(e,g) contains native *Taq* DNA Polymerase in a proprietary formulation. The GoTaq enzyme is supplied with 5X Green and 5X Colorless GoTaq Reaction Buffers. The Green Reaction Buffer contains a compound that increases sample density so that samples sink easily into the wells of an agarose gel. The Green Reaction Buffer also contains two dyes (a blue dye and a yellow dye) that separate during electrophoresis and can be used to monitor migration progress. This allows reactions to be directly loaded onto agarose gels without the need for loading dye. The blue dye migrates at the same rate as 3–5kb DNA fragments in a 1% agarose gel. The yellow dye migrates at a rate faster than primers (<50bp) in a 1% agarose gel. The Colorless GoTaq Reaction Buffer has the same formulation as the Green Reaction Buffer but does not contain dyes. The Colorless Reaction Buffer is recommended for any application where absorbance or fluorescence measurements of the PCR amplimer are necessary before clean-up. Both 5X buffers are supplied at pH 8.5 and contain MgCl₂ at a concentration of 7.5mM, giving a final concentration of 1.5mM in the reaction.

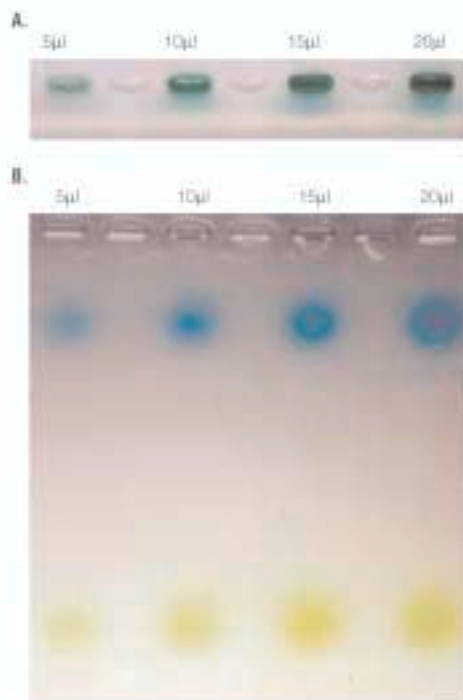
GoTaq® DNA Polymerase

Cat. #: M3001 (100u; 80 reactions)
M3005 (500u; 400 reactions)
M3008 (2,500u; 2,000 reactions)

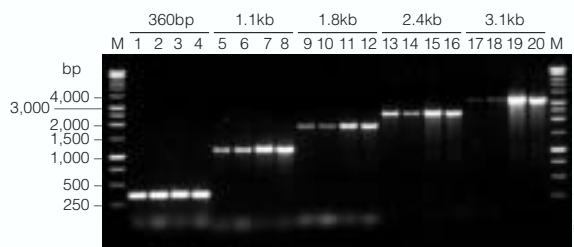
Supplied with enzyme (5u/μl), 5X Green GoTaq Reaction Buffer and 5X Colorless GoTaq Reaction Buffer. Sufficient to give the indicated number of 50μl reactions using 1.25u of enzyme per reaction.

Protocol:

www.promega.com/tbs/9pim300/9pim300.html



Separation of the components of the GoTaq Green Reaction Buffer during electrophoresis. PCR samples amplified using GoTaq DNA Polymerase and GoTaq Green Reaction Buffer were loaded onto an agarose gel. Samples are shown before (A) and after (B) electrophoresis. Volumes indicate the amount of amplification reaction loaded on the gel.



Amplification of various templates with GoTaq DNA Polymerase and other Promega *Taq* DNA Polymerase formulations. The reactions for each template are loaded in this order: *Taq* DNA Polymerase in Storage Buffer B (Cat.# M1661); *Taq* DNA Polymerase in Storage Buffer A (Cat.# M1861); GoTaq DNA Polymerase in Colorless Reaction Buffer; GoTaq DNA Polymerase in Green Reaction Buffer. Reactions without the Green GoTaq Reaction Buffer require the addition of loading dye prior to electrophoresis.

Compatibility of GoTaq DNA Polymerase with Upstream and Downstream Applications.

Product	Cat. #	Green Reaction Buffer	Colorless Reaction Buffer
T-Vector Cloning			
pGEM®-T & pGEM®-T Easy Systems ^(h,i)	A1360, A1380, A3600, A3610	Yes	Yes
pTARGET™ Mammalian Expression Vector System ^(i,j)	A1410	Yes	Yes
PCR Clean-Up			
Wizard® MagneSil® PCR Clean-Up System ^(a)	A1930	Yes	Yes
Wizard® SV 96 PCR Clean-Up System	A9340	Yes	Yes
Wizard® SV Gel and PCR Clean-Up System	A9281	Yes	Yes
Wizard® PCR Preps DNA Purification System ^(k)	A7170	Yes	Yes
Two-Step RT-PCR			
Reverse Transcription System ^(l,m)	A3500	Yes	Yes
ImProm-II™ Reverse Transcription System ^(l,m)	A3800	Yes	Yes
Transcription/Translation			
TNT® T7 Quick for PCR DNA ^(l,m,n)	L5540	Yes	Yes

Amplifying DNA

Routine PCR (continued)

Taq DNA Polymerase & PCR Core Systems: Value and Quality

Promega is a premier supplier of native *Taq* DNA Polymerase. We offer many options for your needs. You can assemble your own reagents from separate *Taq* DNA Polymerase and dNTPs, or purchase the PCR Core Systems to get everything together in one package. The PCR Core Systems^(f) are supplied with a Technical Bulletin that contains thorough coverage of considerations involved in routine PCR amplification and extensive troubleshooting information.

Taq DNA Polymerase in Storage Buffer A

Cat.#: M1861 (100u; 80 reactions)
M1865 (500u; 400 reactions)
M1868 (2,500u; 2,000 reactions)

Supplied with *Taq* DNA Polymerase 10X Reaction Buffer, 25mM MgCl₂. One reaction uses 1.25u of enzyme.

Taq DNA Polymerase in Storage Buffer B

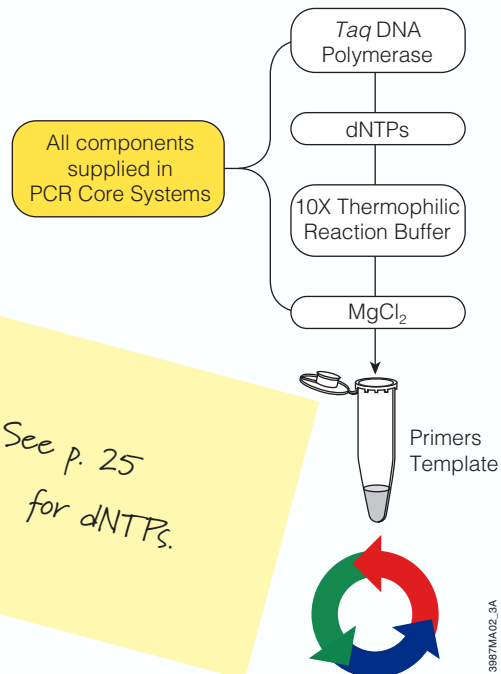
Cat.#: M1661 (100u; 80 reactions)
M1665 (500u; 400 reactions)
M1668 (2,500u; 2,000 reactions)

Supplied with *Taq* DNA Polymerase 10X Reaction Buffer, 25mM MgCl₂. One reaction uses 1.25u of enzyme.

Citations for use of *Taq* DNA Polymerase online at:
www.promega.com/citations/

Taq DNA Polymerase in Storage Buffer A & B

Promega first offered *Taq* DNA Polymerase stabilized with Triton[®] X-100 (*Taq* DNA Polymerase in Storage Buffer A). Later we developed *Taq* DNA Polymerase stabilized with Tween[®]-20 and NP-40 (*Taq* DNA Polymerase in Storage Buffer B). In most cases there is no difference in performance. One key distinction between the two products is compatibility with other suppliers reaction buffers. *Taq* DNA Polymerase in Storage Buffer A must be used with the supplied Reaction Buffer, which contains 0.1% Triton[®] X-100 at the 1X concentration. *Taq* DNA Polymerase in Storage Buffer B does not have this requirement and can be used either with the supplied Promega Reaction Buffer or with other *Taq* DNA polymerase reaction buffers.



The PCR Core Systems are great for researchers just learning PCR

PCR Core System I

Cat.#: M7660 (200 × 50µl reactions; 1.25u *Taq* DNA Polymerase/reaction)

Comes with 250u *Taq* DNA Polymerase in Storage Buffer B, *Taq* DNA Polymerase 10X Reaction Buffer without MgCl₂, *Taq* DNA Polymerase 10X Reaction Buffer with MgCl₂ (1.5mM at 1X), 25mM MgCl₂, PCR Nucleotide Mix.

PCR Core System II

Cat.#: M7665 (200 reactions; 1.25u *Taq* Polymerase/50µl reaction)

Same components as M7660 plus Positive Control Plasmid DNA template and Upstream and Downstream Control Primers.

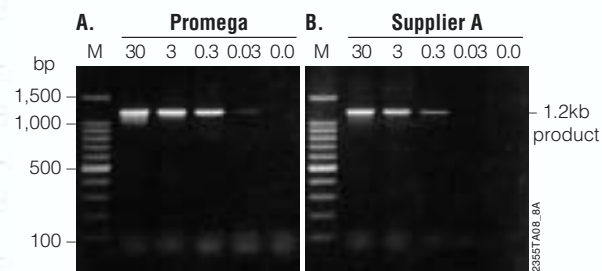
Protocol:

www.promega.com/tbs/tb254/tb254.html

Amplifying DNA

Proofreading Polymerases

Incorporation fidelity can be an important consideration for cloning projects. Thermostable enzymes with a 3'→5' exonuclease activity, commonly known as proofreading activity, offer the highest fidelity in amplification reactions. Proofreading enzymes like *Pfu* and *Tli* DNA Polymerase^(e) offer three- to sixfold higher fidelity than standard *Taq* DNA Polymerase. In general, proofreading enzymes extend primers a little slower than *Taq* DNA Polymerase and thus typically require longer extension times and a few more cycles. Assume 2 minutes per kilobase of amplicon, and add 2–3 cycles to your reaction when using a proofreading enzyme. With *Pfu* DNA Polymerase^(e), it is important to use the Reaction Buffer supplied with the enzyme for maximum fidelity. *Pfu* Reaction Buffer is formulated to give maximum fidelity, not maximum yield. After all, you use *Pfu* for fidelity not yield. If you need greater yield, use more template DNA.



Comparison of sources of native *Pfu* DNA Polymerase. A 1.2kb fragment of human α 1-antitrypsin gene was amplified using *Pfu* DNA Polymerase from Promega (Panel A) and from another supplier (Panel B). The target was amplified from decreasing amounts of Human Genomic DNA (Cat.# G3041) as indicated. Lane M, 100bp DNA Ladder (Cat.# G2101).

Pfu DNA Polymerase

Cat.#: M7741 (100u; 80 reactions)
M7745 (500u; 400 reactions)

Each provided with enzyme (5u/ μ l), *Pfu* DNA Polymerase 10X Reaction Buffer (2mM $MgSO_4$ @ 1X) sufficient to give the indicated number of 50 μ l reactions using 1.25u of enzyme.

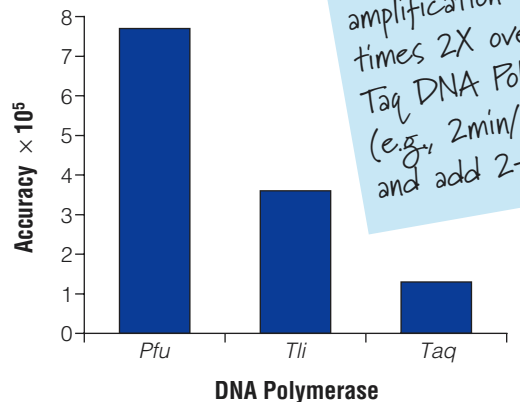
Protocol:

www.promega.com/tbs/9pim774/9pim774.html

Citations for use of *Pfu* DNA Polymerase online at:

www.promega.com/citations/

Not available in North America.



Accuracy of thermostable polymerases. The accuracy of *Pfu* DNA Polymerase has been reported by Cline *et al.* as 7.7×10^5 . Using the PCR-based forward mutation assay, they reported the accuracy of *Pfu* as approximately two-fold higher than Vent[®] (*Tli* DNA Polymerase) and approximately sixfold higher than *Taq* DNA Polymerase (1).

Reference

- Cline, J., Braman, J.C. and Hogrefe, H.H. (1996) PCR fidelity of *Pfu* DNA polymerase and other thermostable DNA polymerases. *Nucl. Acids Res.* **24**, 3546–51.

Proofreaders have a 3'→5' exonuclease activity that is lacking in non-proofreading enzymes like *Taq* DNA Polymerase. Proofreading enzymes can degrade primers if the reaction is allowed to sit for too long prior to amplification. We recommend setting up reactions on ice and adding the proofreading polymerase just prior to placing the reaction into a preheated thermal cycler.

Tli DNA Polymerase

Cat.#: M7101 (50u; 40 reactions)

Supplied with enzyme (5u/ μ l), Thermophilic DNA Polymerase 10X Reaction Buffer and 25mM $MgCl_2$. Sufficient to give the indicated number of 50 μ l reactions using 1.25u of enzyme/reaction.

Citations for use of *Tli* DNA Polymerase online at:

www.promega.com/citations/

Amplifying DNA

Hot Start Methodology

Hot start PCR is a commonly used technique to reduce nonspecific amplification. One cause of nonspecific amplification is the assembly of PCR reactions at room temperature or on ice. Under these conditions, the PCR primers may be able to anneal to various non-complementary positions on the template. Although activity of thermostable DNA polymerases at room temperature or 4°C is usually less than 25%, they can extend nonspecifically annealed primers at these temperatures. Any newly synthesized product is completely complementary to the PCR primer, allowing the primer to anneal specifically to this region during PCR, resulting in an undesired amplification product.

Hot start PCR can also reduce the amount of primer-dimer formed. Primer-dimers result from complementarity between the 3' ends of the PCR primers. At room temperature or on ice, these complementary regions anneal and the polymerase extends the ends to produce a primer-dimer. Primer-dimers often appear as a diffuse band at ~50–100bp on ethidium bromide-stained gels. Both nonspecific products and primer-dimers can compete with the desired amplification reaction for reagents. By avoiding the conditions that lead to nonspecific amplification, hot start PCR can improve the yield of the desired PCR product.

There are several ways to perform hot start PCR. The reaction can be assembled on ice or at room temperature, **omitting** the DNA polymerase until the reaction has been placed in the thermal cycler and heated to 60–65°C. Once the reaction has reached 60–65°C the desired amount of polymerase can be added. This prevents the polymerase from extending primers until the higher temperature is reached and primer annealing is more specific. The method is quite effective but can be labor-intensive, particularly if dozens of amplification reactions are involved.

Another approach to hot start PCR involves the use of wax to physically sequester one or more critical reaction

components until the appropriate temperature is reached. Wax beads can be added to a PCR before the addition of the DNA polymerase. Heating the PCR to 60°C melts the wax, which forms a liquid layer over the surface of the reaction, eliminating the need for mineral oil. Upon cooling to 4°C, the wax solidifies. The DNA polymerase is added onto this wax layer and, as the PCR is heated during the first denaturation step, the wax melts and the polymerase can access the other PCR reagents. This method is labor intensive—requiring an additional heating and cooling step to prepare the wax layer. Also, opening the PCR tube to add the polymerase increases the risk of contamination. Additionally, the solid wax layer that forms upon cooling to 4°C will clog pipet tips when attempting to break through the wax to pipet the PCR. Thus, it is often necessary to use one pipet tip to puncture the wax layer and a second pipet tip to remove the PCR products.

TaqBead Hot Start Polymerase

Enter Promega's *TaqBead* Hot Start Polymerase^(f). By impregnating a wax bead with *Taq* DNA Polymerase, the additional heating and cooling steps to form the wax layer are eliminated. A single bead is added to each 50µl reaction, and as the reaction is heated, the wax melts and releases the polymerase. The molten wax rises to the surface of the PCR where it forms an incomplete barrier. In thermal cyclers with heated lids, a hole remains above the reaction to ease pipetting. To prevent evaporation in thermal cyclers without heated lids, we recommend adding mineral oil to each PCR. The molten wax and mineral oil will mix during the thermal cycling to form a single layer, which solidifies when the reaction is cooled to 4°C and can clog pipet tips.

TaqBead™ Hot Start Polymerase

Cat. #: M5661 (100 reactions)

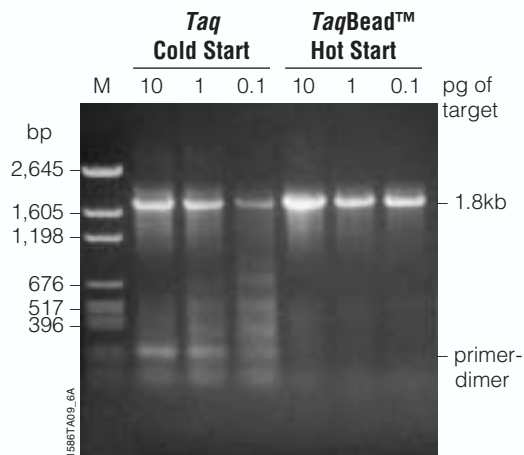
Supplied with 100 beads (1.25µ *Taq* DNA Polymerase in Storage Buffer B/bead), Thermophilic DNA Polymerase 10X Reaction Buffer, and 25mM MgCl₂.

Protocol:

www.promega.com/tbs/tb247/tb247.html

Citations for use of TaqBead Polymerase online at:

www.promega.com/citations/



Hot start amplification reduces the yield of nonspecific amplification products. Aliquots of 10, 1 or 0.1pg pGEM[®]-*Luc* Vector^(h.o.) (Cat.# E1541) were diluted in 30ng of Human Genomic DNA (Cat.# G3041). A 1.8kb luciferase gene product was amplified by PCR using *Taq* DNA Polymerase (Storage Buffer B) or *TaqBead* Hot Start Polymerase in Promega Reaction Buffer supplemented with 2mM MgCl₂. Details are provided in Miller, K., Smith, R. and Storts, D. (1996) Improved PCR amplification using *TaqBead* Hot Start Polymerase. *Promega Notes* 60, 2–6.

Amplifying DNA

dNTPs

Promega is a premier supplier of high-quality dNTPs^(f) in bulk form or premixed in the PCR Nucleotide Mix^(f). Promega's dNTPs are >99% triphosphate with verified concentrations. All dNTPs, whether bulk or premixed, are DNase- and RNase-free, and are functionally tested in amplification reactions. The PCR Nucleotide Mix is also functionally tested in RT-PCR.

Set of dATP, dCTP, dGTP and dTTP

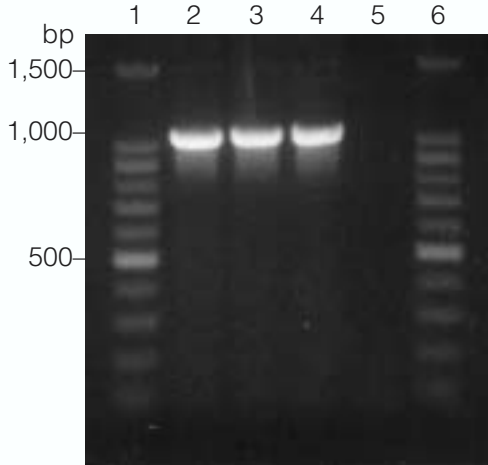
Cat.#: U1330 (10µmol each; 1,000 reactions)
U1420 (25µmol each; 2,500 reactions)
U1240 (40µmol each; 4,000 reactions)
U1410 (200µmol each; 20,000 reactions)

Set of dUTP, dATP, dCTP and dGTP

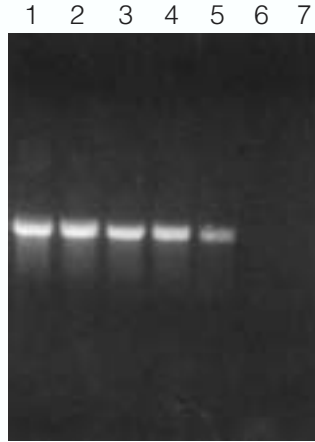
Cat.#: U1335 (10µmol each; 1,000 reactions)
U1245 (40µmol each; 4,000 reactions)

Each dNTP is supplied at 100mM. Reaction size is considered to be 200µM each dNTP in a 50µl PCR reaction.

Custom and bulk dNTP sizes are available.



Stability of dNTPs. The integrity of the RT-PCR products on the gel demonstrates the stability of the dNTPs after storage under the conditions listed. The dNTPs used in each RT-PCR were stored as follows prior to use: Lane 2, 1 freeze-thaw cycle; lane 3, 50 freeze-thaw cycles; lane 4, 1 year at -20°C; lane 5, negative PCR control; lanes 1 and 6, 100bp DNA Ladder (Cat.# G2101).



RT-PCR functional assay using PCR Nucleotide Mix. The dNTPs were used following 50 freeze-thaw cycles. Amounts of template RNA: lane 1, 25fmol; lane 2, 2.5fmol; lane 3, 250amol; lane 4, 25amol; lane 5, 2.5amol; lane 6, 250zmol; lane 7, no template control.

PCR Nucleotide Mix

Cat.#: C1141 (200µl; 200 reactions)
C1145 (1,000µl; 1,000 reactions)

The PCR Nucleotide Mix supplies a single solution containing each dNTP (dATP, dTTP, dGTP, dCTP) at 10mM. Reaction size is considered to be 200µM of each dNTP in a 50µl reaction. Each reaction uses 1µl of PCR Nucleotide Mix.

Custom and bulk PCR Nucleotide Mix sizes are available.

Protocol:

www.promega.com/tbs/9pic114/9pic114.html

Promega's dNTPs and PCR Nucleotide Mix can go through at least 50 freeze/thaws.

Amplifying DNA

Routine PCR

Product	Size	Cat.#
PCR Master Mix ^(f,g)	100 reactions	M7502
	1,000 reactions	M7505

For Laboratory Use. A reaction consists of 25µl of the 2X PCR Master Mix in a 50µl total volume. Supplied with Nuclease-Free Water.

Product	Size	Cat.#
GoTaq [®] DNA Polymerase ^(e,g)	100u	M3001
	500u	M3005
	2,500u	M3008

For Laboratory Use. Supplied with 5X Green GoTaq[®] Reaction Buffer and 5X Colorless GoTaq[®] Reaction Buffer. Both buffers contain 1.5mM Mg²⁺ at the 1X concentration.

Product	Size	Cat.#
PCR Core System I ^(f)	200 reactions	M7660
PCR Core System II ^(f)	200 reactions	M7665

For Laboratory Use. PCR Core Systems provide *Taq* DNA Polymerase in Storage Buffer B, PCR Nucleotide Mix, *Taq* DNA Polymerase 10X Reaction Buffers with and without MgCl₂, and 25mM MgCl₂ sufficient for 200 × 50µl reactions containing 1.25u of *Taq* DNA Polymerase. PCR Core System II also contains Positive Control Plasmid DNA template, and Upstream and Downstream Control Primers.

Product	Size	Cat.#
<i>Taq</i> DNA Polymerase in Storage Buffer A ^(e)	100u	M1861
(Supplied with <i>Taq</i> DNA Polymerase 10X Reaction Buffer without MgCl ₂ , and 25mM MgCl ₂ .)	500u	M1865
	2,500u	M1868
<i>Taq</i> DNA Polymerase in Storage Buffer A ^(e)	100u	M2861
(Supplied with <i>Taq</i> DNA Polymerase 10X Reaction Buffer with MgCl ₂ , giving 1.5mM Mg ²⁺ at the 1X concentration.)	500u	M2865
	2,500u	M2868
<i>Taq</i> DNA Polymerase in Storage Buffer B ^(e)	100u	M1661
(Supplied with <i>Taq</i> DNA Polymerase 10X Reaction Buffer without MgCl ₂ , and 25mM MgCl ₂ Solution.)	500u	M1665
	2,500u	M1668
<i>Taq</i> DNA Polymerase in Storage Buffer B ^(e)	100u	M2661
(Supplied with <i>Taq</i> DNA Polymerase 10X Reaction Buffer with MgCl ₂ , giving 1.5mM Mg ²⁺ at the 1X concentration.)	500u	M2665
	2,500u	M2668

For Laboratory Use.

Proofreading Polymerases

Product	Size	Cat.#
<i>Pfu</i> DNA Polymerase ^{(e)*}	100u	M7741
(Supplied with <i>Pfu</i> DNA Polymerase 10X Reaction Buffer with MgSO ₄ , giving 2mM Mg ²⁺ at the 1X concentration.)	500u	M7745
<i>Tli</i> DNA Polymerase ^{(e)**}	50u	M7101

(Supplied with Thermophilic DNA Polymerase 10X Reaction Buffer and 25mM MgCl₂.)

*Not Available in North America. **For Laboratory Use.

Amplifying DNA

Hot Start Polymerase

Product	Size	Cat.#
TaqBead™ Hot Start Polymerase ^(f) (Supplied with Thermophilic DNA Polymerase 10X Reaction Buffer and 25mM MgCl ₂) For Laboratory Use. One bead per reaction; 1.25u Taq DNA Polymerase per bead.	100 reactions	M5661

dNTPs

Product	Size	Cat.#
PCR Nucleotide Mix ^(f) (Contains 10mM each dNTP; use 1µl per 50µl reaction.)	200µl	C1141
	1,000µl	C1145
Set of dATP, dCTP, dGTP, and dTTP ^(f) (100mM each dNTP. Individual tubes available.)	10µmol	U1330
	25µmol	U1420
	40µmol	U1240
	200µmol	U1410
Set of dUTP, dCTP, dGTP, and dATP ^(f,p) (100mM each dNTP.) For Laboratory Use.	10µmol	U1335
	40µmol	U1245

Accessories

Product	Size	Cat.#
Promega 10 Barrier Tips, 960/pk	0.5–10µl	A1491
Promega 10E Barrier Tips, 960/pk	0.5–10µl	A1501
Promega 10F Barrier Tips, 960/pk	0.5–10µl	A1511
Promega 20 Barrier Tips, 960/pk	2–20µl	A1521
Promega 100 Barrier Tips, 960/pk	10–100µl	A1541
Promega 200 Barrier Tips, 960/pk	50–200µl	A1551
Promega 1000 Barrier Tips, 480/pk	100–1,000µl	A1561
Mineral Oil*	12ml	DY1151
Nuclease-Free Water*	150ml	P1195

*For Laboratory Use.

Tip and Pipette Compatibility Guide

	Size	Pipetman®	Eppendorf®	Oxford Benchmate®	Finnpipette®
Promega 10	0.5–10µl	P-2; P-10		0.5–10µl	0.5–10µl Digital
Promega 10E	0.5–10µl	P-2; P-10	0.5–10µl	0.5–10µl	
Promega 10F	0.5–10µl				0.5–10µl
Promega 20	2–20µl	P-20	2–20µl		
Promega 100	10–100µl	P-100	10–100µl	10–50µl	5–40µl
Promega 200	50–200µl	P-200	EDP-250µl	40–200µl	40–200µl
Promega 1000	100–1,000µl	P-1000		200–1,000µl	200–1,000µl