June 2010

TopTaq™ PCR Handbook

TopTaq DNA Polymerase

TopTaq Master Mix Kit

For standard and specialized end-point PCR applications without the need for optimization

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

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- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

TopTaq DNA Polymerase	**(250 U)**	**(1000 U)**	**(5000 U)**
Catalog no.	**200203**	**200205**	**200207**
TopTaq DNA Polymerase	250 units	1000 units	5000 units
TopTaq PCR Buffer, 10x*	1.2 ml	4 x 1.2 ml	1 x 22 ml
CoralLoad Concentrate, 10x	1.2 ml	4 x 1.2 ml	4 x 5.5 ml
Q-Solution™, 5x	2 ml	4 x 2 ml	1 x 40 ml
$MgCl_2$, 25mM	1.2 ml	4 x 1.2 ml	1 x 22 ml
Handbook	1	1	1

* Contains 15 mM $MgCl_2$

TopTaq Master Mix Kit	
Catalog no.	**200403**
Number of units	**250**
Number of 50 µl reactions	**200**
TopTaq Master Mix†, 2x	3 x 1.7 ml
CoralLoad Concentrate, 10x	1 x 1.2 ml
RNase-Free Water	3 x 1.9 ml
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† Contains TopTaq DNA Polymerase, TopTaq PCR Buffer with 3 mM $MgCl_2$ and 400 µM each dNTP.

Shipping and Storage

TopTaq DNA Polymerase and the TopTaq Master Mix Kit are shipped on dry ice but retain full activity at room temperature (15–25°C) for at least 2 weeks. They should be stored immediately upon receipt at 2–8°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance. TopTaq DNA Polymerase and the TopTaq Master Mix Kit can also be stored at –20°C in a constant-temperature freezer at least until the expiration date (see the inside of the kit lid).

Product Use Limitations

TopTaq DNA Polymerase and TopTaq Master Mix Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding TopTaq DNA Polymerase Kit, TopTaq Master Mix Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Product Specifications

Enzyme:

TopTaq DNA Polymerase is a recombinant thermostable 94 kDa DNA polymerase.

Concentration:	5 units/µl
Compatible substrate analogs:	dNTP, ddNTP
Extension rate:	2–4 kb/min at 72°C
5'–3' exonuclease activity:	Yes
Extra A addition:	Yes
3'–5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No
Storage and dilution buffer:	20 mM Tris·Cl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (v/v), stabilizers; pH 9.0 (20°C).

Buffers and reagents:

TopTaq PCR Buffer:	10x concentrated. Contains Tris·Cl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, stabilizers; pH 8.7 (20°C).
CoralLoad Concentrate:	10x concentrated. Contains gel loading reagent, orange dye, red dye.

Q-Solution:	5x concentrated
$MgCl_2$ Solution:	25 mM
TopTaq Master Mix:	2x concentrated. Contains TopTaq DNA Polymerase, PCR Buffer (with 3 mM $MgCl_2$), and 400 µM each dNTP.

Quality Control

Enzyme:	(see quality-control label inside kit lid for lot-specific values.)
Amplification efficiency assay:	The amplification efficiency is tested in parallel amplification reactions and is indicated under "Amp".
PCR reproducibility assay:	PCR reproducibility and specificity are tested in parallel amplification reactions. The reactions must yield a single specific product.
Exonuclease activity assay:	Linearized plasmid DNA is incubated with TopTaq DNA Polymerase in PCR Buffer. Exonuclease activity per unit of enzyme is indicated under "Exo".
Endonuclease activity assay:	Plasmid DNA is incubated with TopTaq DNA Polymerase in PCR Buffer. Endonuclease activity per unit of enzyme is indicated under "Endo".
RNase activity assay:	RNA is incubated with TopTaq DNA Polymerase in PCR Buffer. RNase activity per unit of enzyme is indicated under "RNase".
Protease activity assay:	TopTaq DNA Polymerase is incubated in storage buffer. Protease activity per unit of enzyme is indicated under "Protease".
Self-priming activity assay:	Assays are performed under standard PCR conditions, without primers, TopTaq DNA Polymerase and human genomic DNA (purified with the QIAamp® DNA Blood Mini Kit). The absence of PCR product is indicated by "No" under "Self priming".

Buffers and Reagents:

PCR Buffer, 10x:	Conductivity, pH, sterility, and performance in PCR are tested.
CoralLoad Concentrate, 10x:	Conductivity, pH, sterility, dye concentrations, and performance in PCR are tested.
Q-Solution, 5x:	Conductivity, pH, sterility, and performance in PCR are tested.
$MgCl_2$, 25 mM:	Conductivity, pH, sterility, and performance in PCR are tested.

TopTaq Master Mix Kit:

PCR reproducibility assay:	PCR reproducibility and specificity are tested in parallel amplification reactions. The reactions must yield a single specific product.

Introduction

TopTaq DNA Polymerase has been developed by QIAGEN to provide highly reliable end-point PCR with unrivalled ease of use. Until now, all PCR enzymes required storage at –20°C; however, due to the unique proprietary TopTaq Stabilizer contained in the enzyme storage buffer, TopTaq DNA Polymerase and TopTaq Master Mix Kits are the first PCR kits that can be stored routinely at 4°C. This results in significant time savings as thawing of reagents is not required. Furthermore, all components can be combined at room temperature eliminating the need for working on ice. The unique buffer formulation and a single pre-optimized protocol eliminate the need for optimization of experimental parameters for individual primer–template systems.

The optional addition of CoralLoad Concentrate to the PCR reaction enables direct loading of the PCR products onto agarose gels without the need to add gel loading buffer, saving time and resources.

The TopTaq Master Mix Kit offers all of the benefits of TopTaq DNA Polymerase combined with the advantage of a ready-to-use master mix. With the TopTaq Master Mix Kit, separate pipetting of individual components is not required and the risk of contamination and pipetting variability is minimized, making PCR setup quick and easy.

TopTaq DNA Polymerase

TopTaq DNA Polymerase is a recombinant 94 kDa thermostable DNA Polymerase. TopTaq DNA Polymerase provides high PCR product yield and increased specificity without the need for optimization.

TopTaq Stabilizer binds to TopTaq DNA Polymerase at 4°C and room temperature, preventing polymerase denaturation during long-term storage. Template DNA and primers are also prevented from binding to the polymerase at low temperature. During the initial denaturation step, the TopTaq Stabilizer dissociates from the polymerase without compromising polymerase activity.

TopTaq Master Mix

TopTaq Master Mix consists of a ready-to-use premixed solution containing TopTaq DNA Polymerase, dNTPs, and the innovative TopTaq PCR Buffer. Providing all components in a ready-to-use master mix reduces pipetting steps, therefore reducing the risk of contamination. High yields of PCR product are achieved, even after storing the TopTaq Master Mix for 4 months at 25°C, 4°C, or –20°C.

CoralLoad Concentrate

TopTaq DNA Polymerase and TopTaq Master Mix Kit are supplied with CoralLoad Concentrate, which contains a gel loading reagent and two gel tracking dyes that facilitate estimation of DNA migration distance and optimization of agarose gel run time. When using CoralLoad Concentrate, the PCR products can be directly loaded onto an agarose gel without prior addition of loading buffer.

CoralLoad dyes do not interfere with most downstream enzymatic applications. However, for reproducible results, purification of PCR products prior to enzymatic manipulation is recommended.

PCR Buffer

The innovative TopTaq PCR Buffer facilitates the amplification of specific PCR products. During the annealing step of every PCR cycle, the buffer greatly increases the ratio of specific primer binding over nonspecific primer binding.

Owing to a uniquely balanced combination of KCl and $(NH_4)_2SO_4$, the PCR buffer provides stringent primer-annealing conditions over a wider range of temperatures and Mg^{2+} concentrations than conventional PCR buffers. The need to optimize PCR by varying the annealing temperature or the Mg^{2+} concentration is dramatically reduced, or often not required.

Q-Solution

TopTaq DNA Polymerase is supplied together with Q-Solution, an innovative PCR additive that facilitates amplification of difficult templates by modifying the melting behavior of DNA. This unique reagent will often enable or improve a suboptimal PCR caused by templates that have a high degree of secondary structure or that are GC-rich. Unlike other commonly used PCR additives such as DMSO, Q-Solution is used at just one working concentration, it is nontoxic, and PCR purity is guaranteed. For further information, please read the protocol “PCR Using TopTaq DNA Polymerase and Q-Solution”, page 18.

Pre-optimized protocol and cycling program

To reduce the time and effort needed for the optimization of experimental parameters, the protocol offers convenient pre-optimized primer concentrations and an annealing temperature that has been shown to work for a large variety of primer–template systems. For optimal results and for your convenience, it is recommended to start with these pre-optimized values.

The PCR cycling program has also been pre-optimized to work for most primer–template systems. For optimal results, please follow the cycling protocol provided in this handbook.

Specificity and sensitivity

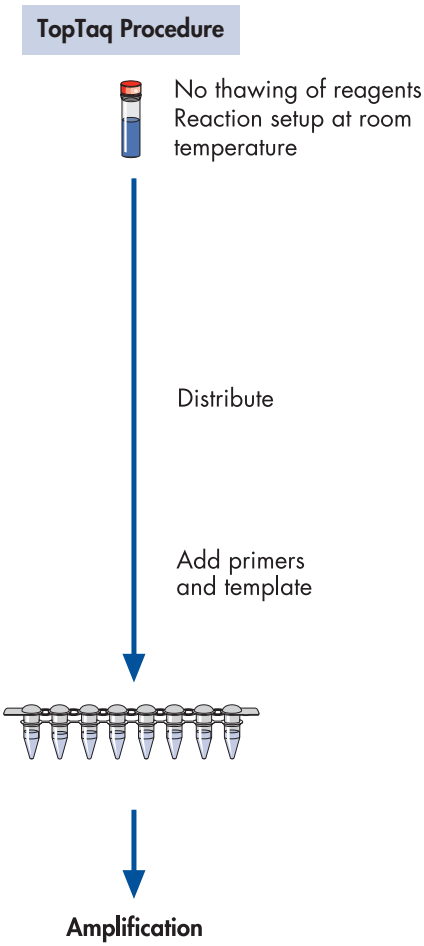
With its balanced potassium and ammonium ions, the unique PCR Buffer used in combination with TopTaq DNA Polymerase and TopTaq Master Mix Kit promotes specific primer–template annealing and simultaneously reduces nonspecific annealing. Maximum yields of specific products are obtained even when using low template amounts.

Downstream applications

TopTaq DNA Polymerase and the TopTaq Master Mix Kit are ideally suited for a wide variety of applications.

For high-fidelity PCR we recommend the HotStar HiFidelity Polymerase Kit for highly sensitive and reliable high-fidelity PCR without optimization.

For hot-start PCR we recommend HotStarTaq® *Plus* DNA Polymerase for maximum specificity without optimization requirements. For even more convenience, we offer HotStarTaq *Plus* Master Mix, which contains a premixed solution for fast and highly specific hot-start PCR amplification.


TopTaq Procedure
No thawing of reagents
Reaction setup at room temperature
Distribute
Add primers and template
Amplification

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- RNase-free water
- Reaction tubes
- Pipets and pipet tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)
- Primers should be purchased from an established oligonucleotide manufacturer, such as Operon Biotechnologies (www.operon.com). Lyophilized primers should be dissolved in TE to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry. Primer stock solutions should be stored in aliquots at –20°C.

Protocol: PCR Using TopTaq DNA Polymerase

Important points before starting

- The protocol offers convenient pre-optimized primer concentrations and an annealing temperature that works for most primer–template systems. It is recommended to start with these pre-optimized values.
- It is essential that the provided 10x TopTaq PCR Buffer is used to ensure optimal PCR performance.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- When downstream applications require fluorescence or absorbance measurements, addition of CoralLoad Concentrate is not recommended unless an intermediate purification of the PCR product (e.g., using QIAquick® or MinElute® PCR Purification Kits) will be performed.

Things to do before starting

- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C. High-quality, PCR-grade dNTP mix (10 mM) is available from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw dNTP mix and primer solutions at room temperature or on ice. Remove TopTaq DNA Polymerase, TopTaq PCR Buffer, and CoralLoad Concentrate from storage at 4°C.**

   It is important to mix all the solutions — especially the TopTaq PCR Buffer—completely before use to avoid localized concentrations of salts.

2. **Prepare a master mix according to Table 1 (page 15).**

   For most primer–template systems, it is not necessary to keep reaction vessels on ice. Due to the unique buffer formulation, TopTaq DNA Polymerase reaction mix exhibits significantly reduced polymerase activity at room temperature.

   The master mix contains all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included.

Note: The Mg^{2+} concentration of 15 mM provided by the supplied 10x TopTaq PCR Buffer will produce satisfactory results in most cases. However, in rare cases, reactions may be improved by increasing the final Mg^{2+} concentration (see Troubleshooting Guide, page 27).

Table 1. Recommended reaction composition using TopTaq DNA Polymerase

Component	Volume/reaction	Final concentration
Master mix		
10x TopTaq PCR Buffer*	5 µl	1x
dNTP mix (10 mM of each)	1 µl	200 µM of each dNTP
Optional: 10x CoralLoad Concentrate	5 µl	1x
Primer A	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
Primer B	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
TopTaq DNA Polymerase	0.25 µl	1.25 units/reaction
RNase-free water	Variable	–
Template DNA		
Template DNA	Variable	≤1 µg/reaction
Total volume	**50 µl**	**–**

Note: If smaller reaction volumes are used, please reduce the amount of each component accordingly.

* Contains 15 mM $MgCl_2$. TopTaq DNA Polymerase should only be used in combination with 10x TopTaq PCR Buffer.

3. **Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes.**

   Mix gently (e.g., by pipetting the master mix up and down a few times). It is not necessary to keep PCR tubes on ice, as the unique PCR buffer formulation prevents nonspecific DNA synthesis at room temperature.

4. **Add template DNA (<1 µg/reaction) to the individual tubes containing the master mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume (see Appendix D, page 35).

5. **When using a thermal cycler with a heated lid, proceed directly to step 6. Otherwise, overlay each reaction with approximately 50 µl mineral oil.**
6. **Program the thermal cycler according to the manufacturer's instructions.**

   The PCR cycling program outlined below has been pre-optimized for most primer–template systems. However, if optimization of the annealing temperature is required, please refer to Appendix B, page 31.

Table 2. Pre-optimized cycling protocol

			Additional comments
Initial denaturation:	**3 min**	**94°C**	
3-step cycling:			
Denaturation:	30 s	94°C	
Annealing:	30 s	60°C	The annealing temperature of **60°C** is suitable for most PCR systems. If optimization of annealing temperature is required, see Table 12, page 32 (Appendix B) for more information.
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 35.
Final extension:	10 min	72°C	

7. **Place the PCR tubes in the thermal cycler and start the cycling program.**

   Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.
8. **When using CoralLoad Concentrate, the PCR reaction can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel tracking dyes.**

   CoralLoad Concentrate contains a gel loading reagent and two gel tracking dyes. Refer to Table 3 for equivalent DNA migration distances at different agarose gel percentages.

Table 3. Migration distances of gel tracking dyes

%TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 bp (270 bp)	~80 bp (<10 bp)
1.0	300 bp (220 bp)	~40 bp (<10 bp)
1.5	250 bp (120 bp)	~20 bp (<10 bp)
2.0	100 bp (110 bp)	<10 bp (<10 bp)
3.0	50 bp (100 bp)	<10 bp (<10 bp)

Protocol: PCR Using TopTaq DNA Polymerase and Q-Solution

This protocol is designed for using Q-Solution in PCR assays. Q-Solution changes the melting behavior of DNA and can be used for PCR systems that do not work well under standard conditions. When using Q-Solution the first time for a particular primer–template pair, always perform parallel reactions with and without Q-Solution. This recommendation should also be followed if another PCR additive (such as DMSO) was previously used for a particular primer–template pair.

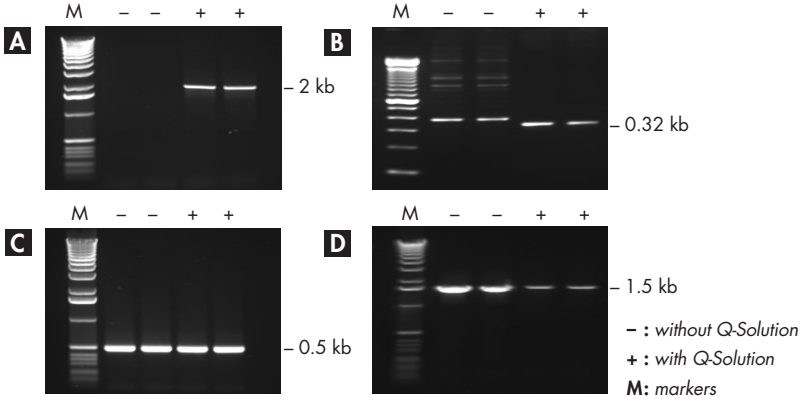
When using Q-Solution, depending on the individual PCR assay, the following effects may be observed:

Case A: Q-Solution enables amplification of a reaction which previously failed.

Case B: Q-Solution increases PCR specificity in certain primer–template systems.

Case C: Q-Solution has no effect on PCR performance.

Case D: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction. In this case, addition of Q-Solution disturbs the previously optimal primer–template annealing. Therefore, when using Q-Solution for the first time for a particular primer–template system, always perform reactions in parallel with and without Q-Solution.



Important points before starting

- When using Q-Solution for the first time in a particular primer–template system, it is important to perform parallel amplification reactions with and without Q-Solution.
- The protocol offers convenient pre-optimized primer concentrations and an annealing temperature that works for most primer–template systems. It is recommended to start with these pre-optimized values.
- It is essential that the provided 10x TopTaq PCR Buffer is used to ensure optimal PCR performance.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- When downstream applications require fluorescence or absorbance measurements, addition of CoralLoad Concentrate is not recommended unless an intermediate purification of the PCR product (e.g., using QIAquick PCR Purification Kits or MinElute PCR Purification Kits) will be performed.

Things to do before starting

- If required, prepare a dNTP mix containing 10 mM of each dNTP. Store this mix in aliquots at –20°C. High-quality, PCR-grade dNTP mix (10 mM) is available from QIAGEN (cat. no. 201900).

Procedure

1. **Thaw dNTP mix and primer solutions at room temperature or on ice. Remove TopTaq DNA Polymerase, TopTaq PCR Buffer, CoralLoad Concentrate, and Q-Solution from storage at 4°C.**

   It is important to mix all the solutions — especially TopTaq PCR Buffer —completely before use to avoid localized concentrations of salts.

   When using Q-Solution, additional $MgCl_2$ is not usually required.

2. **Prepare a master mix at room temperature according to Table 4 (page 20).**

   For most primer–template systems, it is not necessary to keep reaction vessels on ice. Due to the unique buffer formulation, TopTaq DNA Polymerase reaction mix exhibits significantly reduced polymerase activity at room temperature.

   The master mix contains all the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included.

Table 4. Recommended reaction composition using TopTaq DNA Polymerase and Q-Solution

Component	Volume/reaction	Final concentration
Master mix		
10x TopTaq PCR Buffer*	5 µl	1x
Optional: 10x CoralLoad Concentrate	5 µl	1x
5x Q-Solution	10 µl	1x
dNTP mix (10 mM of each)	1 µl	200 µM of each dNTP
Primer A	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
Primer B	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
TopTaq DNA Polymerase	0.25 µl	1.25 units/reaction
RNase-free water	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	**50 µl**	**–**

Note: If smaller reaction volumes are used, please reduce the amount of each component accordingly.

* Contains 15 mM $MgCl_2$. TopTaq should only be used in combination with 10x TopTaq PCR buffer.

3. **Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes.**

   Mix gently (e.g., by pipetting the master mix up and down a few times). It is not necessary to keep PCR tubes on ice, as the unique PCR buffer formulation prevents nonspecific DNA synthesis at room temperature.

4. **Add template DNA (<1 µg/reaction) to the individual tubes containing the master mix.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume (see Appendix D, page 35).

5. **When using a thermal cycler with a heated lid, proceed directly to step 6. Otherwise, overlay each reaction with approximately 50 µl mineral oil.**

6. **Program the thermal cycler according to the manufacturer's instructions.**

   The PCR cycling program outlined below has been pre-optimized for most primer–template systems. However, if an optimization of the annealing temperature is required, please refer to Appendix B, Page 31.

Table 5. Pre-optimized cycling protocol

			Additional comments
Initial denaturation:	**3 min**	**94°C**	
3-step cycling:			
Denaturation:	30 s	94°C	
Annealing:	30 s	60°C	The annealing temperature of **60°C** is suitable for most PCR systems. If optimization of annealing temperature is required, see Table12, page 32 (Appendix B) for more information.
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 35.
Final extension:	10 min	72°C	

7. **Place the PCR tubes in the thermal cycler and start the cycling program.**

   Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.

8. **When using CoralLoad Concentrate, the PCR reaction can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel tracking dyes.**

   CoralLoad Concentrate contains a gel loading reagent and two gel tracking dyes. Refer to Table 6 below for equivalent DNA migration distances at different agarose gel percentages.

Table 6. Migration distances of gel tracking dyes

%TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 bp (270 bp)	~80 bp (<10 bp)
1.0	300 bp (220 bp)	~40 bp (<10 bp)
1.5	250 bp (120 bp)	~20 bp (<10 bp)
2.0	100 bp (110 bp)	<10 bp (<10 bp)
3.0	50 bp (100 bp)	<10 bp (<10 bp)

Protocol: PCR Using TopTaq Master Mix Kit

Important points before starting

- This protocol offers convenient, pre-optimized primer concentrations and an annealing temperature that works for most primer–template systems. It is recommended to start with these pre-optimized values.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- CoralLoad Concentrate is not recommended when downstream applications require fluorescence or absorbance measurements without an intermediate purification of the PCR product (e.g., using QIAquick PCR Purification Kits or MinElute PCR Purification Kits).

Procedure

1. **Thaw primer solutions and template DNA.**

   Mix well before use.

2. **Mix the TopTaq Master Mix by vortexing briefly and dispense 25 µl into each PCR tube according to Table 7.**

   It is important to mix the TopTaq Master Mix before use in order to avoid localized concentrations of salt. For most primer-template systems, it is not necessary to keep reaction vessels on ice since TopTaq Master Mix exhibits significantly reduced polymerase activity at room temperature due to the unique buffer formulation.

3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the Master Mix.**

4. **Add template DNA (1 µg /reaction) to the individual PCR tubes.**

   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume (see Appendix D, page 35).

Table 7. Recommended reaction composition using TopTaq Master Mix

Component	Volume/reaction	Final concentration
TopTaq Master Mix, 2x	25 µl	1.25 units TopTaq DNA Polymerase 1 x PCR Buffer* 200 µM of each dNTP
Diluted primer mix		
Primer A	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
Primer B	Variable	0.1–0.5 µM; **0.2 µM** is suitable for most PCR systems
Optional: CoralLoad Concentrate, 10x	5 µl	1x CoralLoad Concentrate
RNase-free water	Variable	–
Template DNA		
Template DNA	Variable	<1µg / reaction
Total volume	**50 µl**	**–**

Note: If smaller or larger reaction volumes are used, please adjust the amount of each component accordingly.

* Contains 1.5 mM $MgCl_2$.

5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.**
6. **Program the thermal cycler according to the manufacturer's instructions.**

   The PCR cycling program outlined below has been pre-optimized for most primer–template systems.

   However, if an optimization of the annealing temperature is required, please refer to Appendix B, Page 31.

Table 8. Pre-optimized cycling protocol

			Additional comments
Initial denaturation:	**3 min**	**94°C**	
3-step cycling:			
Denaturation:	30 s	94°C	
Annealing:	30 s	60°C	The annealing temperature of **60°C** is suitable for most PCR systems with TopTaq Master Mix. If optimization of annealing temperature is required, see Table 12, page 32 (Appendix B) for more information.
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		See Appendix C, page 35.
Final extension:	10 min	72°C	

7. **Place the PCR tubes in the thermal cycler and start the cycling program.**

   Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.

8. **When using CoralLoad Concentrate, the PCR reaction can be directly loaded onto an agarose gel without prior addition of a PCR loading buffer and gel tracking dyes.**

   CoralLoad PCR Buffer contains a gel loading reagent and gel tracking dyes. Please refer to Table 9 below to identify the dyes according to migration distance and agarose gel percentage and type.

Table 9. Migration distances of gel tracking dyes

%TAE (TBE) agarose gel	Red dye	Orange dye
0.8	500 bp (270 bp)	~80 bp (<10 bp)
1.0	300 bp (220 bp)	~40 bp (<10 bp)
1.5	250 bp (120 bp)	~20 bp (<10 bp)
2.0	100 bp (110 bp)	<10 bp (<10 bp)
3.0	50 bp (100 bp)	<10 bp (<10 bp)

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Little or no product		
a)	Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of reagents, including primers and dNTP mix.
b)	Wrong PCR buffer	The 10x TopTaq PCR Buffer provided in the kit is required for the optimal performance.
c)	PCR cycling conditions are not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol on page 18.
d)	Primer concentration not optimal or primers degraded	The primer concentration of 0.2 µM is suitable for most primer–template systems with TopTaq DNA Polymerase. However, when primer concentration optimization is desired, repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM steps). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.*
e)	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template (see Appendix A, page 30). If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

		Comments and suggestions
f)	Mg^{2+} concentration not optimal	Perform PCR with different final concentrations of Mg^{2+} from 1.5–5.0 mM (in 0.5 mM steps) using a 25 mM $MgCl_2$ solution.
g)	Enzyme concentration too low	When using TopTaq DNA Polymerase, use 1.25 units per 50 µl reaction. If necessary, increase the amount of TopTaq DNA Polymerase (in 0.5 unit steps).
h)	Insufficient number of cycles	Increase the number of cycles in steps of 5 cycles (see Appendix C, page 35).
i)	Incorrect annealing temperature or time	Decrease annealing temperature in 2°C steps. Annealing time should be between 30 and 60 s. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix E, page 37).
j)	Incorrect denaturation temperature or time	Denaturation should be at 94°C for 30 to 60 s. Ensure that the initial 3 minute 94°C incubation step is performed as described in step 6 of the PCR protocols (pages 16, 21, and 24).
k)	Extension time too short	Increase the extension time in increments of 1 min.
l)	Primer design not optimal	Review primer design (see Appendix B, page 31).
m)	RT reaction error	For RT-PCR, take into consideration the efficiency of the reverse transcriptase reaction, which averages 10–30%. The added volume of reverse transcriptase reaction should not exceed 10% of the final PCR volume (see Appendix D, page 35).
n)	PCR overlaid with mineral oil when using a thermal cycler with a heated lid	When performing PCR in a thermal cycler with a heated lid, do not overlay the PCR samples with mineral oil if the heated lid is switched on as this may decrease the yield of PCR product.
o)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.

		Comments and suggestions
Product is multi-banded		
a)	PCR cycling conditions not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol on page 18.
b)	Annealing temperature too low	Increase annealing temperature in 2°C steps. Annealing time should be between 30 and 60 s. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix E, page 37).
c)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer in 0.1 µM steps). In particular, when performing highly sensitive PCR check for possible degradation of the primers on a denaturing polyacrylamide gel.*
d)	Primer design not optimal	Review primer design (see Appendix B, page 31).
Product is smeared		
a)	Too much starting template	Check the concentration and storage conditions of the starting template (see Appendix A, page 30). Make serial dilutions of template nucleic acid from stock solutions. Perform PCR using these serial dilutions. When re-amplifying a PCR product, start the re-amplification round using 1 µl of a 1-in-103 – 104 dilution of the previous PCR.
b)	Carryover contamination	If the negative-control PCR (without template DNA) shows a PCR product or a smear, exchange all reagents. Use disposable pipet tips containing hydrophobic filters to minimize cross-contamination. Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

		Comments and suggestions
c)	Enzyme concentration too high	Use 1.25 units TopTaq DNA Polymerase per 50 µl reaction. When using TopTaq Master Mix, always use 25 µl of TopTaq Master Mix per 50 µl reaction.
d)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.
e)	Mg^{2+} concentration not optimal	Perform PCR with different final concentrations of Mg^{2+} from 1.5–5.0 mM (in 0.5 mM steps) using the 25 mM $MgCl_2$ solution provided.
f)	Primer concentration not optimal or primers degraded	The primer concentration of 0.2 µM is suitable for most PCR systems with TopTaq DNA Polymerase. However, when primer concentration optimization is desired, repeat the PCR with different primer concentrations from 0.1–0.5 µM of each primer (in 0.1 µM steps). In particular, when performing highly sensitive PCR check for possible degradation of the primers on a denaturing polyacrylamide gel.*
g)	Primer design not optimal	Review primer design (see Appendix B, page 31).

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix A: Starting Template

Both the quality and quantity of nucleic acid starting template affect PCR, in particular the sensitivity and efficiency of amplification.*

Quality of starting template

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, ethanol, EDTA, and other chemical solvents than single-step enzyme-catalyzed processes. QIAGEN offers a complete range of nucleic acid preparation systems, ensuring the highest-quality templates for PCR. These include the QIAprep® system for rapid plasmid purification, the QIAamp and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids, and the RNeasy® system for RNA preparation from a variety of sources. For more information about QIAprep, QIAamp, DNeasy, and RNeasy products, contact one of our Technical Service Departments (see back cover) or visit www.qiagen.com/productfinder .

Quantity of starting template

The annealing efficiency of primer to template is an important factor in PCR. Owing to the thermodynamic nature of the reaction, the primer: template ratio strongly influences the specificity and efficiency of PCR and should be optimized empirically. If too little template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mispriming events. As an initial guide, spectrophotometric and molar conversion values for different nucleic acid templates are listed in Tables 10 and 11, respectively.

Table 10. Spectrophotometric conversions for nucleic acid templates

1 A_{260} unit†	Concentration (µg/ml)
Double-stranded DNA	50
Single-stranded DNA	33
Single-stranded RNA	40

† Absorbance at 260 nm = 1

* For further information see our guide Critical Factors for Successful PCR. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Table 11. Molar conversions for nucleic acid templates

Nucleic acid	Size	pmol/µg	Molecules/µg
1 kb DNA	1000 bp	1.52	9.1×10^{11}
pUC19 DNA	2686 bp	0.57	3.4×10^{11}
pTZ18R DNA	2870 bp	0.54	3.2×10^{11}
pBluescript® II DNA	2961 bp	0.52	3.1×10^{11}
Lambda DNA	48,502 bp	0.03	1.8×10^{10}
Average mRNA	1930 nt	1.67	1.0×10^{12}
Genomic DNA			
Escherichia coli	4.7×10^{6}*	3.0×10^{-4}	1.8×10^{8}†
Drosophila melanogaster	1.4×10^{8}*	1.1×10^{-5}	6.6×10^{5}†
Mus musculus (mouse)	2.7×10^{9}*	5.7×10^{-7}	3.4×10^{5}†
Homo sapiens (human)	3.3×10^{9}*	4.7×10^{-7}	2.8×10^{5}†

* Base pairs in haploid genome.

† For single-copy genes.

Appendix B: Primer Design, Concentration, and Storage

Standard PCR primers

Prerequisites for successful PCR include the design of optimal primer pairs, the use of appropriate primer concentrations, and the correct storage of primer solutions. Some general guidelines are given in Table 12.‡

‡ For further information see our guide Critical Factors for Successful PCR. To obtain a copy, visit the QIAGEN web site at www.qiagen.com or call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Table 12. General guidelines for standard PCR primers

Length:	18–30 nucleotides
G/C content:	40–60%
T_m:	Simplified formula for estimating melting temperature (T_m): T_m = 2°C x (A+T) + 4°C x (G+C) Whenever possible, design primer pairs with similar T_m values. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m. If the recommended annealing temperature of 60°C (mentioned in the protocol on pages 16, 21 and 24) does not give satisfactory results, please carry out a temperature gradient PCR with annealing temperatures from 50–68°C to identify the optimum annealing temperature.
Sequence:	■ Avoid complementarity of two or three bases at the 3' ends of primer pairs to reduce primer–dimer formation. ■ Avoid mismatches between the 3' end of the primer and the target-template sequence. ■ Avoid runs of 3 or more G or C at the 3' end. ■ Avoid a 3'-end T. Primers with a T at the 3' end have a greater tolerance of mismatch. ■ Avoid complementary sequences within a primer sequence and between the primer pair. ■ Commercially available computer software (e.g., Primer Designer 1.0, Scientific Software, 1990; Oligo, Rychlik, and Rhoads, 1989) can be used for primer design.

Concentration:

- Spectrophotometric conversion for primers: 1 A_{260} unit = 20–30 µg/ml
- Molar conversions:

Primer length	pmol/µg	20 pmol
18mer	168	119 ng
20mer	152	132 ng
25mer	121	165 ng
25mer	121	165 ng
30mer	101	198 ng

- Use 0.1–0.5 µM of each primer in PCR. For most applications, a primer concentration of 0.2 µM will be sufficient.

Storage:

Lyophilized primers should be dissolved in a small volume of distilled water or TE to make a concentrated stock solution.

Prepare small aliquots of working solutions containing 10 pmol/µl to avoid repeated thawing and freezing. Store all primer solutions at –20°C. Primer quality can be checked on a denaturing polyacrylamide gel;* a single band should be seen.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Degenerate PCR primers

Occasionally, the exact nucleotide sequence of the target-template DNA will not be known, for instance when it has been deduced from an amino acid sequence. To enable such templates to be amplified by PCR, degenerate primers can be used. These are actually mixtures of several primers whose sequences differ at the position that correspond to the uncertainties in the template sequence.

PCR using TopTaq Master Mix often improves the specificity of PCR amplifications that employ degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. Table 13 gives recommendations for further optimizing PCR using degenerate primers.

Table 14 shows the codon redundancy of each amino acid.

Table 13. Guidelines for design and use of degenerate primers

Sequence:	■ Avoid degeneracy in the 3 nucleotides at the 3' end. ■ If possible, use Met- or Trp-encoding triplets at the 3' end. ■ To increase primer–template binding efficiency, reduce degeneracy by allowing mismatches between the primer and template, especially towards the 5' end (but not at the 3' end). ■ Try to design primers with less than 4-fold degeneracy at any given position.
Concentration:	■ Begin PCR with a primer concentration of 0.2 µM. ■ In case of poor PCR efficiency, increase primer concentrations in increments of 0.25 µM until satisfactory results are obtained.

Table 14. Codon redundancy

Amino acid	Number of codons
Met, Trp	1
Cys, Asp, Glu, Phe, His, Lys, Asn, Gln, Tyr	2
Ile	3
Ala, Gly, Pro, Thr, Val	4
Leu, Arg, Ser	6

Appendix C: Number of PCR Cycles

A cycling program usually consists of between 25 and 35 cycles, depending on the number of copies of the starting template. Too many cycles do not necessarily lead to a higher yield of PCR product; instead they may increase nonspecific background and decrease the yield of specific PCR product.

Table 15 provides a general guideline for choosing the number of cycles.

Table 15. General guidelines for choosing the number of PCR cycles

Number of copies of starting template*	1 kb DNA	*E. coli* DNA†	Human genomic DNA†	Number of cycles
10–100	0.01–0.11 fg	0.05–0.56 pg	36–360 pg	40–45
100–1000	0.11–1.1 fg	0.56–5.56 pg	0.36–3.6 ng	35–40
$1 \times 10^3 - 5 \times 10^4$	1.1–55 fg	5.56–278 pg	3.6–179 ng	30–35
$>5 \times 10^4$	>55 fg	>278 pg	>179 ng	25–35

* Refer to Table 11 (page 31) to calculate the number of molecules. When starting with cDNA templates, it is important to take into account the efficiency of reverse transcription in cDNA synthesis, which is on average 10–30%.

† Refers to single-copy genes.

Appendix D: RT-PCR

To perform PCR using RNA as a starting template, the RNA must first be reverse transcribed into cDNA in a reverse transcriptase reaction (RT reaction). Failure of the subsequent PCR is often a result of the limitations of the RT reaction. On average, only 10–30% of the original RNA molecules are reverse transcribed into cDNA. The expression level of the target RNA molecules and the relatively low efficiency of the reverse transcription reaction must be considered when calculating the appropriate amount of starting template for subsequent PCR. The volume of the RT reaction transferred should not exceed 10% of the total PCR volume. General guidelines are presented in Table 16, page 36.

Table 16. General guidelines for performing RT-PCR

RNA purification and reverse transcription:	QIAGEN offers the RNeasy system for total RNA isolation, Oligotex® Kits for messenger RNA isolation, and Omniscript® Reverse Transcriptase for reverse transcription.* Follow the detailed protocol in the *Omniscript Reverse Transcriptase Handbook*. When using an enzyme from another supplier, follow the manufacturer's instructions. The following guidelines may be helpful. ■ Mix the following reagents in a microcentrifuge tube: 4.0 µl 5x RT buffer 1.0 µl RNase inhibitor (5 units/µl) 2.0 µl DTT (0.1 M) 1.0 µl each dNTP (10 mM) ~1 µg RNA 2.5 µl primer (0.2 µg/µl) Reverse transcriptase† Add RNase-free water to a final volume of 20 µl. ■ Incubate following the manufacturer's instructions. ■ Heat the reaction mix to 95°C for 5 min to inactivate the reverse transcriptase.
PCR:	■ Prepare a PCR mixture following steps 1–3 in protocols. ■ Add 2–5 µl from the RT reaction to each PCR tube containing the master mix. ■ Continue with step 5 in the PCR protocols.

Oligotex resin is not available in Japan.

* For further information about RNeasy, Oligotex, and Omniscript products, contact your local QIAGEN Technical Services or distributor (see back cover) or visit www.qiagen.com .

† Please refer to the manufacturer's instructions for the amount of enzyme required.

Appendix E: Touchdown PCR

Touchdown PCR uses a cycling program with varying annealing temperatures. It is a useful method to increase the specificity of PCR. The annealing temperature in the initial cycle should be 5–10°C above the T_m of the primers. In subsequent cycles, the annealing temperature is decreased in steps of 1–2°C/cycle until a temperature is reached that is equal to, or 2–5°C below, the T_m of the primers. Touchdown PCR enhances the specificity of the initial primer–template duplex formation and hence the specificity of the final PCR product.

To program your thermal cycler for touchdown PCR, you should refer to the manufacturer's instructions.

Appendix F: Purification of PCR Products

After amplification, the PCR sample contains a complex mixture of specific PCR product and residual reaction components such as primers, unincorporated nucleotides, enzyme(s), salts, mineral oil, and probably nonspecific amplification products. Before the specific PCR product can be used in subsequent experiments it is often necessary to remove these contaminants. The QIAquick system offers a quick and easy method for purifying the final PCR product. Using the MinElute system, PCR products can be purified in higher concentrations due to the low elution volumes needed in this system. Gel loading reagent and tracking dyes are effectively removed with the QIAquick and MinElute systems. For more information about QIAquick and MinElute products, please call QIAGEN Technical Services or your local distributor (see back cover) or visit www.qiagen.com .

Appendix G: Control of Contamination

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

General physical precautions

- Separate the working areas for setting up the PCR master mix and DNA handling, including the addition of starting template, PCR product analysis, or plasmid preparation. Ideally, use separate rooms.
- Use a separate set of pipets for the PCR master mix. Use of pipet tips with hydrophobic filters is strongly recommended.
- Prepare and freeze small aliquots of primer solutions and dNTP mix. Use of fresh distilled water is strongly recommended.
- In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution.*† Afterwards, the benches and pipets should be rinsed with distilled water.

General chemical precautions

- PCR stock solutions can also be decontaminated using UV light. This method is laborious, however, and its efficiency is difficult to control and cannot be guaranteed. We recommend storing solutions in small aliquots and using fresh aliquots for each PCR.
- Another approach to preventing amplification of contaminating DNA is to treat individual reaction mixtures with DNase I or restriction enzymes that cut between the binding sites of the amplification primers used, before adding the template DNA sample.

* Most commercial bleach solutions are approximately 5.25% sodium hypochlorate.

† When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Ordering Information

Product	Contents	Cat. no.
TopTaq DNA Polymerase (250)	250 units TopTaq DNA Polymerase*, 10x PCR Buffer,† 10x CoralLoad Concentrate, 5x Q-Solution, 25 mM $MgCl_2$	200203
TopTaq DNA Polymerase (1000)	4 x 250 units TopTaq DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad Concentrate, 5x Q-Solution, 25 mM $MgCl_2$	200205
TopTaq DNA Polymerase (5000)	5000 units TopTaq DNA Polymerase*, 10x PCR Buffer,† 10x CoralLoad Concentrate, 5x Q-Solution, 25 mM $MgCl_2$	200207
TopTaq Master Mix Kit (250)	3 x 1.7 ml 2x TopTaq Master Mix‡ containing 250 units TopTaq DNA Polymerase in total, 1x 1.2 ml 10x CoralLoad Concentrate, 3 x 1.9 ml RNase-Free Water, reagents provided for 200 x 50 µl reactions	200403
Related products		
HotStarTaq Plus DNA Polymerase — for highly specific hot-start PCR without optimization		
HotStarTaq *Plus* DNA Polymerase (250 U)§	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,† 10x CoralLoad PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203603

* Supplied in a single tube.

† Contains 15 mM $MgCl_2$.

‡ Contains 3 mM $MgCl_2$ and 400 µM each dNTP

§ Larger kit sizes available; see www.qiagen.com .

Ordering Information

Product	Contents	Cat. no.
HotStarTaq *Plus* Master Mix Kit — for fast and highly specific amplification		
HotStarTaq *Plus* Master Mix Kit (250)*	3 x 0.85 ml HotStarTaq *Plus* Master Mix,† containing 250 units of HotStarTaq *Plus* DNA Polymerase total, 1 x 0.55 ml CoralLoad Concentrate, 2 x 1.9 ml RNase-Free Water for 250 x 20 µl reactions.	203643
HotStar HiFidelity Polymerase Kit — for highly sensitive and reliable high-fidelity hot-start PCR		
HotStar HiFidelity Polymerase Kit (100 U)*	100 units HotStar HiFidelity DNA Polymerase, 5x HotStar HiFidelity PCR Buffer (inc. dNTPs),‡ 5x Q-Solution, 25 mM $MgSO_4$, RNase-Free Water	202602
QIAGEN Fast Cycling PCR Kit — for rapid and highly specific PCR on any thermal cycler		
QIAGEN Fast Cycling PCR Kit (200)*	2 x 1 ml QIAGEN Fast Cycling PCR Master Mix, 10 x CoralLoad Dye, Q-Solution, RNase-Free Water suitable for 200 x 20 µl reactions.	203743
dNTP Set and dNTP Mix, PCR Grade — for sensitive and reproducible PCR and RT-PCR		
dNTP Mix, PCR Grade (200 µl)*	Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (1 x 200 µl)	201900
dNTP Set, PCR Grade, 4 x 100 µl*	100 mM each dATP, dCTP, dGTP, dTTP for 1000 x 50 µl PCR reactions	201912

* Larger kit sizes available; see www.qiagen.com .

† Contains 15 mM $MgCl_2$.

‡ Contains Factor SB, dNTPs, and optimized concentration of $MgSO_4$.

Ordering Information

Product	Contents	Cat. no.
QIAxcel System — for effortless automated DNA fragment and RNA analysis		
QIAxcel System	Capillary electrophoresis device, including computer, and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
QIAxcel kits — for fast high-resolution capillary electrophoresis		
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Large Fragment Kit (600)	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
MinElute PCR Purification Kit — for purification of PCR products (70 bp to 4 kb) in low elution volumes		
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
QIAquick PCR Purification Kit — for purification of PCR products, 100 bp to 10 kb		
QIAquick PCR Purification Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick Gel Extraction Kit — for gel extraction or cleanup of DNA (70 bp to 10 kb) from enzymatic reactions		
QIAquick Gel Extraction Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704

* Larger kit sizes available; see www.qiagen.com .

Ordering Information

Product	Contents	Cat. no.
dNTP Set and dNTP Mix, PCR Grade — for sensitive and reproducible PCR and RT-PCR		
dNTP Mix, PCR Grade (200 µl)*	Mix containing 10 mM each of dATP, dCTP, dGTP, and dTTP (1 x 200 µl)	201900
dNTP Set, PCR Grade, 4 x 100 µl*	100 mM each dATP, dCTP, dGTP, dTTP for 1000 x 50 µl PCR reactions	201912

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