January 2018

AllTaq® PCR Core and Master Mix Kits Handbook

For ultrafast and highly sensitive hot-start PCR

Sample to Insight

QIAGEN

Contents

Kit Contents

AllTaq PCR Core Kit	**(250)**	**(1000)**	**(5000)**
Cat. no.	**203123**	**203125**	**203127**
AllTaq DNA Polymerase (5U/µl)	50 µl	200 µl	5 x 200 µl
AllTaq PCR Buffer, 5x	1.2 ml	2 x 1.2 ml	9 x 1.2 ml
Q-Solution®, 5x	2 ml	2 ml	5 x 2 ml
dNTP Mix (10 mM each)	55 µl	200 µl	5 x 200 µl
$MgCl_2$, 25 mM	1.2 ml	1.2 ml	2 x 1.2 ml
PCR Template Tracer, 25x	200 µl	200 µl	4 x 200 µl
PCR MM Tracer, 125x	50 µl	2 x 50 µl	9 x 50 µl
RNase-Free Water	1.9 ml	4 x 1.9 ml	16 x 1.9 ml
Quick-Start Protocol	1	1	1

AllTaq Master Mix Kit	**(500)**	**(2500)**
Cat. no.	**203144**	**203146**
AllTaq Master Mix, 4x, containing: AllTaq PCR Buffer, AllTaq DNA Polymerase and dNTP-Mix	2 x 1.25 ml	10 x 1.25 ml
PCR Template Tracer, 25x	200 µl	5 x 200 µl
PCR MM Tracer, 125x	2 x 50 µl	10 x 50 µl
RNase-Free Water	5 x 1.9 ml	20 x 1.9 ml
Quick-Start Protocol	1	1

Storage

The AllTaq PCR Core Kit and the AllTaq Master Mix Kit are shipped on dry ice and should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer. When the kits are stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the kit label). The Template Tracer and Master Mix Tracer can also be stored at 2–8°C until the expiration date. The AllTaq PCR Master Mix can also be stored at 2–8°C for up to 6 months, depending on the expiration date.

If desired, the PCR Master Mix Tracer can be added to AllTaq PCR Buffer (5x) or the AllTaq Master Mix (4x) for long-term storage. For details, see “Adding Master Mix Tracer to the PCR Mix”, page 10.

Intended Use

The AllTaq PCR Core Kit and the AllTaq Master Mix Kit are intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AllTaq PCR Core Kit and AllTaq Master Mix Kit is tested against predetermined specifications to ensure consistent product quality.

Product Information

The AllTaq PCR Core Kit contains:

Component	Description
AllTaq DNA Polymerase	AllTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. AllTaq DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 2-minute, 95°C incubation step or a 3-minute, 93°C incubation step.
AllTaq PCR Buffer, 5x	Contains Tris·HCl, KCl, NH_4SO_4, $MgCl_2$ and additives enabling fast cycling and direct loading of the reactions onto agarose gels.
PCR Master Mix Tracer	Orange dye allows tracking of master mix addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 50 bp on a 1% agarose gel.
PCR Template Tracer	Blue dye allows tracking of template DNA addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 4000 bp on a1% agarose gel.
Q-Solution, 5x	5x concentrated
$MgCl_2$ Solution	25 mM
RNase-Free Water	Ultrapure quality, PCR-grade
dNTP mix (10 mM each)	Contains dATP, dCTP, dGTP and dTTP of ultrapure quality.

The AllTaq Master Mix Kit contains:

Component	Description
AllTaq Master Mix, 4x	Contains AllTaq PCR Buffer and additives enabling fast cycling and direct loading of the reactions onto agarose gels. Also contains AllTaq DNA Polymerase and dNTP-Mix.
PCR Master Mix Tracer	Orange dye allows tracking of master mix addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 50 bp on a 1% agarose gel.
PCR Template Tracer	Blue dye allows tracking of template DNA addition and monitoring of agarose gel loading and electrophoresis. The dye runs at approximately 4000 bp on a 1% agarose gel.
RNase-Free Water	Ultrapure quality, PCR-grade.

Introduction

The AllTaq PCR Core Kit and the AllTaq Master Mix Kit provide a convenient format for highly sensitive and specific hot-start PCR using any DNA/cDNA template. The features of both kits render them ideal for all PCR applications. Features include: visual pipetting controls, a fast cycling protocol, extreme room-temperature stability during and after reaction setup, and a 4x concentrated master mix format, allowing for a higher sample input volume.

The AllTaq PCR Core Kit comprises the following components:

AllTaq PCR Buffer

- The innovative AllTaq PCR Buffer facilitates the amplification of specific PCR products. During the annealing step of every PCR cycle, the buffer allows a high ratio of specific-to-nonspecific primer binding. The verified buffer composition is adapted to ultra-fast cycling conditions and simultaneously provides stringent primer-annealing conditions over a wide range of annealing temperatures. It also ensures perfect duplex capabilities. Optimization of PCR by varying the annealing temperature or the Mg^{2+} concentration is not required.

AllTaq DNA Polymerase

- AllTaq DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. At low temperatures, AllTaq DNA polymerase is kept in an inactive state by an antibody and a novel guard additive, which stabilizes the complex. This improves the stringency of the hot-start and prevents any enzymatic activity at ambient temperature. The enzyme is fully activated after the 2-minute incubation step at 95°C and starts amplifying with high specificity from the first cycle.
- The hot-start procedure eliminates extension from nonspecifically annealed primers and primer–dimers in the first cycle, ensuring highly specific and reproducible PCR.

- The hot-start mechanism enables complete reaction set up at room temperature. After reaction setup, samples can be left at room temperature for more than 3 days before being subjected to PCR cycling.

Q-Solution

- The AllTaq PCR Core Kit is provided with Q-Solution, an additive that facilitates amplification of difficult templates by modifying the melting behavior of nucleic acids.
- Q-Solution often enables or improves suboptimal PCR caused by DNA templates that have a high degree of secondary structure or that are GC-rich.
- Unlike other commonly used additives, for example DMSO, Q-Solution is used at just one working concentration. For further information, please read "AllTaq PCR using Q-Solution", page 21.

The AllTaq Master Mix Kit comprises the following components:

- The AllTaq Master Mix is a suitable solution to reduce hands-on time, due to its pre-mixed core components, containing: AllTaq DNA Polymerase, AllTaq PCR buffer and dNTPs, rendering the Master Mix ready to use. The 4x concentration allows greater flexibility for template input volume over common 2x concentrations.

Other components in the AllTaq PCR Core Kit and the AllTaq Master Mix Kit:

PCR Master Mix Tracer and PCR Template Tracer

- The blue and orange dyes in the PCR Template Tracer and in the PCR Master Mix Tracer, respectively, allow visual tracking of pipetted samples during the PCR setup, to prevent errors. When the blue template is added to the orange PCR Master Mix, the color changes to green, confirming that sample was added. The use of these tracers is

optional. The blue PCR Template Tracer is provided as a 25x concentrate and should be diluted to obtain a 1x final concentration in the sample*.

- The orange PCR Master Mix Tracer is provided as a 125x concentrate and can be added directly to the master mix stock vial to obtain a 1x final concentration. These tracers do not reduce sample stability or PCR performance.
- Reactions can be directly loaded onto agarose gels after cycling. Each tracer dye allows monitoring of the loading process and efficient tracking of the subsequent electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

AllTaq PCR Core Kit and the AllTaq Master Mix procedure

The AllTaq PCR Core Kit and the AllTaq Master Mix Kit allows fast and easy PCR setup. Whatever your application – virus detection, molecular diagnostics research, or gene expression – just mix all components together in one tube and start your thermal cycler program (see Figure 1).

* Example: add 0.2 µl of the blue Template Tracer (25x) to 5 µl sample before use. If pipetting volumes are too small to handle, the Template Tracer can be pre-diluted using sterile water. In this example, 2 µl of 1:10 pre-diluted Template Tracer could be added

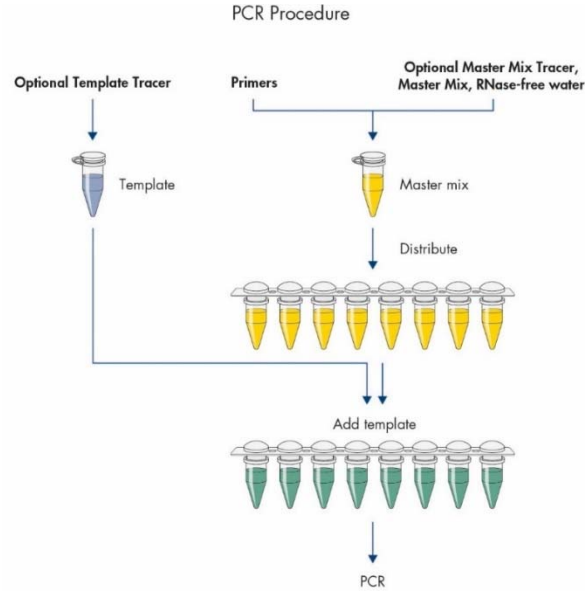

Figure 1. AllTaq procedure using tracer dyes.

Adding Master Mix Tracer to the PCR Buffer and Master Mix

The orange PCR Master Mix Tracer can be added directly to the AllTaq PCR buffer or the AllTaq Master Mix for long-term storage. Since the amount of tracer added is very small, the concentration of the master mix will not be changed and the master mix can be used as indicated in the protocols.

Table 1. Addition of PCR Master Mix Tracer to the AllTaq PCR buffer or the AllTaq Master Mix

Volume of 5x AllTaq PCR Buffer	Volume of 125x PCR Master Mix Tracer
1.2 ml	48 µl
Volume of 4x AllTaq Master Mix	Volume of 125x PCR Master Mix Tracer
1.25 ml	40 µl

Equipment and Reagents to Be Supplied by User

The AllTaq PCR Core Kit and the AllTaq Master Mix Kit are designed to be used with gene-specific primers.

- Microcentrifuge tubes or PCR strips
- PCR tubes or plates
- LoBind tubes (e.g., from AXYGEN® or Eppendorf®)
- Thermocycler
- Microcentrifuge
- Vortexer
- Pipettes and pipette tips

Protocol: Using AllTaq PCR Core Kit

Important points before starting

- The protocol has been optimized for 0.1 pg–1 µg of total DNA.
- AllTaq PCR Kits are designed for use with primers at a final concentration of 0.25 µM.
- AllTaq DNA Polymerase requires a heat-activation step of 2 min at 95°C, or for amplicons longer than 1kbp, 3 min at 93°C.
- It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of AllTaq DNA Polymerase.
- The AllTaq PCR Core Kit is provided with Q-Solution, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.
- AllTaq PCR buffer in the AllTaq PCR Core Kit contains an optimized concentration of Mg^{2+}, which typically does not require adjustment. If addition of more Mg^{2+} is desired, the optimal concentration should be determined empirically by adding provided $MgCl_2$ solution in steps of 0.5 mM, corresponding to 0.5 µl in a 25 µl reaction.

Procedure

1. When using the AllTaq PCR Core Kit, thaw AllTaq PCR Buffer, dNTP-Mix, template DNA or cDNA, primer solutions, RNase-free water, $MgCl_2$ (optional), Template Tracer (optional), MM Tracer (optional) and 5x Q-Solution (optional).

   Mix thoroughly before use by vortexing.

2. Prepare a reaction mix according to Table 2. The reaction mix contains all the components except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed. It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template) should be included in every experiment.

Table 2. Reaction setup for the AllTaq PCR Core Kit

Component	Volume/reaction	Final concentration
Reaction mix AllTaq PCR Buffer, 5x	5 µl	1x
AllTaq DNA Polymerase	0.5 µl	2.5 U/rxn
dNTP-Mix, 10 mM each	0.5 µl	0.2 mM
Primer A	Variable	0.25 µM
Primer B	Variable	0.25 µM
RNase-free water	Variable	–
Optional: $MgCl_2$, 25 mM	Variable	–
Optional: Master Mix Tracer,125x	0.2 µl	1x
Optional: 5x Q-Solution*	5 µl	1x
Template DNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	25 µl†	

*For templates with GC-rich regions or complex secondary structure.

† For PCR in a 384-well plate, we recommend a final reaction volume of 10 µl. Reduce pipetting volumes accordingly.

3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times or vortexing a few seconds. Dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template DNA (1 µg – 100 fg per reaction, depending on target abundance) to the individual PCR tubes. The AllTaq PCR Core Kit and the AllTaq Master Mix Kit can be used with genomic DNA, cDNA, plasmid DNA, oligonucleotides and other DNA molecules as template. If using cDNA as template, the input volume from undiluted cDNA reaction should not exceed 10% of the total PCR reaction volume.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Tables 3 and 4.

Table 3. AllTaq cycling conditions for amplicons ≤ 1 kbp

Step	Time	Temperature	Comments
Initial PCR activation	2 min	95°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	5 s	95°C	Do not exceed this temperature.
Annealing	15 s	55°C	Approximately 5°C below Tm of primers.
Extension	10 s	72°C	For PCR products up to 1000 bp, an extension time of 10 s is sufficient.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

Table 4. AllTaq cycling conditions for amplicons >1-9* kbp

Step	Time	Temperature	Comments
Initial PCR activation	3 min	93°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	15 sec	93°C	Do not exceed this temperature.
Annealing	30 sec	60°C	Approximately identical to Tm of primers.
Extension	1 min/kb	68°C	Allow 1min per kbp amplicon size.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

*Performance in amplification of long targets depends on the quality of the template and assay.

6. Place the PCR tubes or plates in the thermal cycler and start the PCR program.

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

7. We have evaluated several specialized protocols and particular hints. See pages 21–35.

Protocol: Using AllTaq Master Mix Kit

Important points before starting

- The protocol has been optimized for 0.1 pg–1 µg of total DNA.
- AllTaq PCR Kits are designed to be used with a final primers concentration of 0.25 µM.
- AllTaq DNA Polymerase requires a heat-activation step of 2 min at 95°C, or for amplicons longer than 1kbp, 3 min at 93°C.
- It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of AllTaq DNA Polymerase.

Procedure

1. When using the Alltaq Master Mix, thaw AllTaq Master Mix, template DNA or cDNA, primer solutions, RNase-free water, Template Tracer (optional) and Master Mix Tracer (optional).

   Mix thoroughly before use by vortexing.

2. Prepare a reaction mix according to Table 5. The reaction mix contains all the components except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed. It is not necessary to keep samples on ice during reaction setup or while programming the cycler.

   Note: A negative control (without template) should be included in every experiment.

Table 5. Reaction setup for the AllTaq Master Mix Kit

Component	Volume/reaction	Final concentration
Reaction mix AllTaq Master Mix, 4x	5 µl	1x
Primer A	Variable	0.25 µM
Primer B	Variable	0.25 µM
RNase-Free Water	Variable	–
Optional: Master Mix Tracer, 125x	0.16 µl	1x
Template DNA (added at step 4)	Variable	0.1 pg – 1 µg/reaction
Total reaction volume	20 µl*	

*For PCR in a 384-well plate, we recommend a final reaction volume of 10 µl. Reduce pipetting volumes accordingly.

3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times or vortexing a few seconds. Dispense appropriate volumes into PCR tubes or wells of a PCR plate.
4. Add template DNA (1 µg – 100 fg per reaction, depending on target abundance) to the individual PCR tubes. The AllTaq PCR Core Kit and the AllTaq Master Mix Kit can be used with genomic DNA, cDNA, plasmid DNA, oligonucleotides and other DNA molecules as template. If using cDNA as template, the input volume from undiluted cDNA reaction should not exceed 10% of the total PCR reaction volume.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Tables 6 and 7.

Table 6. AllTaq cycling conditions for amplicons ≤ 1 kbp

Step	Time	Temperature	Comments
Initial PCR activation	2 min	95°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	5 sec	95°C	Do not exceed this temperature.
Annealing	15 sec	55°C	Approximately 5°C below Tm of primers.
Extension	10 sec	72°C	For PCR products up to 1000 bp, an extension time of 10 s is sufficient.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

Table 7. AllTaq cycling conditions for amplicons >1-9* kbp

Step	Time	Temperature	Comments
Initial PCR activation	3 min	93°C	This heating step activates AllTaq DNA Polymerase.
3-step cycling:			
Denaturation	15 sec	93°C	Do not exceed this temperature.
Annealing	30 sec	60°C	Approximately identical to Tm of primers.
Extension	1 min/kb	68°C	Allow 1min per kbp amplicon size.
Number of cycles	40		The optimal cycle number depends on the amount of template and the abundance of the target.

*Performance in amplification of long targets depends on the quality of the template and assay.

6. Place the PCR tubes or plates in the thermal cycler and start the PCR program.

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

7. We have evaluated several specialized protocols and particular hints. See pages 21–35.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Little or no product		
a)	Pipetting error or missing reagent	Check the concentrations and storage conditions of reagents, including primers. Repeat the PCR.
b)	DNA polymerases not activated	Ensure that the cycling program included the DNA polymerase activation step (2 min at 95°C or 3 min at 93° C) as described in the cycling protocols.
c)	Primer concentration not optimal or primers degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.2–1.0 µM in 0.1 µM increments.
d)	PCR conditions not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol described in Appendix A on page 21.
e)	Problems with starting template	Check the concentration, integrity, purity and storage conditions of the starting template (see Appendix D, page 26). If necessary, make new serial dilutions of template DNA from stock solutions. Repeat the PCR using the new dilutions.
f)	Mg^{2+} concentration not optimal	Perform PCR with different final concentrations of Mg^{2+} by adding $MgCl_2$ in 0.5 mM step to the reaction, using the 25 mM $MgCl_2$ solution provided in the AllTaq PCR Core Kit. Do not exceed 3 mM additional $MgCl_2$.
g)	Insufficient number of cycles	Increase the number of cycles in increments of 5 cycles.
h)	Incorrect PCR annealing temperature or time	Decrease annealing temperature in 2°C steps. Annealing time should be between 15 and 30 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix F, page 34).
i)	Incorrect denaturation temperature or time	Denaturation should be at 95°C for 5 seconds. Ensure that the cycling program included the DNA polymerase activation step (2 min at 95°C) as described in the cycling protocols. If amplifying PCR products longer than 1 kbp, decrease denaturation temperature to 93°C and increase denaturation time to 15 seconds.

		Comments and suggestions
i)	Insufficient starting template	Increase the template amount. If this is not possible, perform a second round of PCR using a nested-PCR approach (see Appendix F, page 34)
k)	Primer design not optimal	Review primer design (see Appendix E, page 33). Only use gene-specific primers.
l)	PCR of long fragments	Increase the concentration of template DNA. When amplifying products longer than 1 kbp, use the modified reaction conditions described in Tables 4 and 7.
m)	Reactions overlaid with mineral oil when using a thermal cycler with a heated lid	When using a thermal cycler with a heated lid that is switched on, do not overlay the reactions with mineral oil as this may decrease the yield of PCR product.
n)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been programmed correctly.
o)	Enzyme concentration too low	When using AllTaq DNA Polymerase, use 2.5 units per 25 µl reaction. If necessary, increase the amount of AllTaq DNA Polymerase (in 0.5 unit steps). When using AllTaq Master Mix, use 5 µl Master Mix per 20 µl reaction.
p)	Extension time too short	Increase the extension time in increments of 10 seconds. For PCR amplicons larger than 1 kbp, follow Tables 4 and 7 "AllTaq cycling conditions for amplicons >1–9 kbp")
Product is multibanded		
a)	PCR cycling conditions not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol in Table 2, page 13.
b)	PCR annealing temperature too low	Increase annealing temperature in increments of 2°C. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR (see Appendix F, page 34).
c)	Primer concentration not optimal or primers degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.25–1.0 µM in 0.1 µM increments.
d)	Primer design not optimal	Review primer design (see Appendix E, page 33). Use only gene-specific primers.
e)	Contamination with genomic DNA	Pretreat starting cDNA template with DNase I. Alternatively, use primers located at splice junctions of the target mRNA to avoid amplification from genomic DNA (see Appendix E, page 33).
Product is smeared		
a)	Too much starting template	Check the concentration of the starting template (see Appendix D, page 26). If necessary, make new serial dilutions of template DNA from stock solutions. Repeat the PCR using the new dilutions.

		Comments and suggestions
b)	Carry-over contamination	If the negative control (without template) 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.
c)	PCR cycling conditions not optimal	Using the same cycling conditions, repeat the PCR using Q-Solution. Follow the protocol in Table 2 on page 13.
d)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.
e)	Primer concentration not optimal or primers degraded	A primer concentration of 0.25 µM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the PCR with different primer concentrations from 0.25–1.0 µM in 0.1 µM increments. In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.
f)	Primer design not optimal	Review primer design (see Appendix E, page 33). Use only gene-specific primers.
g)	Enzyme concentration too high	When using AllTaq DNA Polymerase, use 2.5 units per 25 µl reaction.

Appendix A: PCR Using Q-Solution

This protocol is designed for use with Q-Solution in PCR for the AllTaq PCR Core Kit. Q-Solution changes the melting behavior of nucleic acids and can be used for PCR systems that do not work well under standard conditions. When using Q-Solution for the first time with a particular primer–template system, always perform parallel reactions with and without Q-Solution.

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

Case A: Q-Solution increases PCR specificity in certain primer–template systems. Q-Solution enables PCR that previously failed.

Case B: Q-Solution causes reduced efficiency or failure of a previously successful amplification reaction.

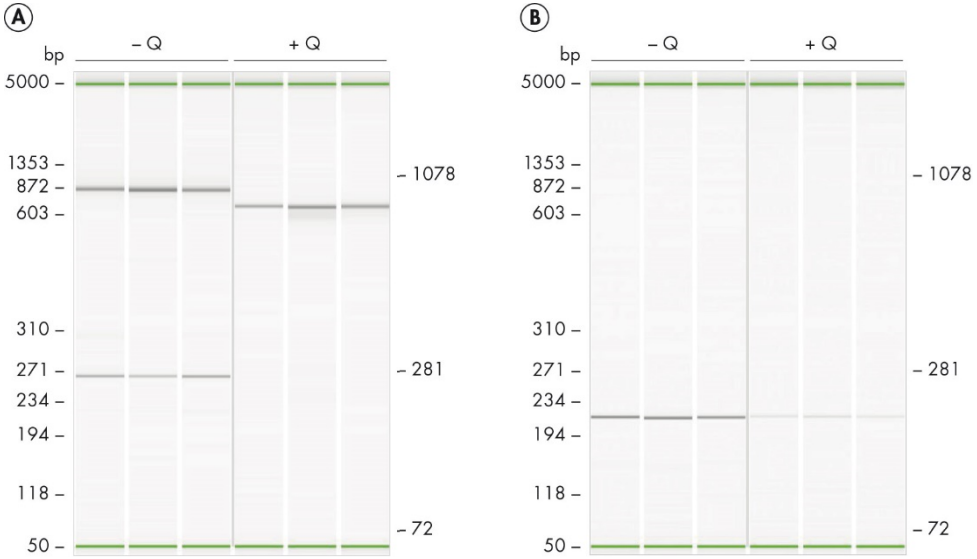

Figure 2. Effects using Q-Solution.

For reaction setup and cyling protocol of the AllTaq PCR Core Kit see Tables 2–4.

Appendix B: Duplex PCR

Duplex PCR allows the simultaneous detection of two different DNA targets in a single reaction. Duplex PCR could be necessary when using a coamplified internal or external control. The AllTaq PCR Core Kit and the AllTaq Master Mix Kit are optimized for singleplex and duplex PCR. Primers should be designed according to the guidelines given below.

Step 1 Select optimal primer pairs

- Choose similar amplicon sizes for both targets, but ensure that the products can be easily distinguished by your read-out system, e.g., gel or capillary electrophoresis.
- Keep amplicon sizes ideally below 300 bp or clearly below 1 kb.

Step 2 Perform duplex PCR

- Modify corresponding reaction setup in Tables 2 and 5 by adding a second primer pair
- Use a primer concentration of 0.25 µM.
- An abundance difference of <3000 and size difference of <400 bp is possible without adapting the primer concentration.
- There is no need for adaption of the cycling protocol. Use the protocol in Table 3.

Step 3 Optimize duplex PCR

- If the PCR from step 2 results in different product yields, reduce the concentration of the primers yielding the most prominent PCR product(s) in steps of 0.1 µM until all products are produced in similar quantities. Concentrations as low as 0.05 µM may be sufficient to amplify abundant targets.
- If altering the primer concentrations fails to improve the yield of long PCR products, increase the extension time in increments of 10 s.

Appendix C: 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, for example the QIAprep® system for rapid plasmid purification, the QIAamp® and DNeasy® systems for rapid purification of genomic DNA and viral nucleic acids. For more information about QIAprep, QIAamp and DNeasy products, contact one of our Technical Service Departments.

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 8 and 9.

Table 8. 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.

Table 9. 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 D: 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 10. The AllTaq PCR Kit is designed to be used with gene-specific primers only.

Table 10. Guidelines for handling and storing primers

	Description
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 PCR annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m .
Location	• If detecting mRNA after conversion into cDNA, design primers so that one half of the primer hybridizes to the 3' end of one exon and the other half to the 5' end of the adjacent exon (see Figure 3A, page 29). Primers will anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA. Thus, amplification of contaminating DNA is eliminated. • Alternatively, PCR primers should be designed to flank a region that contains at least one intron (see Figure 3B, page 29). Products amplified from cDNA (no introns) will be smaller than those amplified from genomic DNA (containing introns). Size difference in products is used to detect the presence of contaminating DNA. • If only the mRNA sequence is known, choose primer annealing sites that are at least 300–400 bp apart. It is likely that fragments of this size from eukaryotic DNA contain splice junctions. As explained in the previous point and Figure 3B, such primers may be used to detect DNA contamination.
Sequence	• Avoid complementarity of two or more 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 nucleotides 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 primers of a primer pair.
- Commercially available computer software 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	12.5 pmol (0.25 µM in 25 µl)	10 pmol (0.25 µM in 20 µl)
18mer	168	37 ng	30 ng
20mer	152	42 ng	34 ng
25mer	121	52 ng	41 ng
30mer	101	62 ng	50 ng

- Use 0.25–1.0 µM of each primer in PCR. For most applications, a primer concentration of 0.25 µM will be optimal.

Storage

- Lyophilized primers should be dissolved in a small volume of TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to make a concentrated stock solution. Prepare small aliquots of working solutions containing10 pmol/µl to avoid repeated thawing and freezing. Store all primer solutions at –20°C.

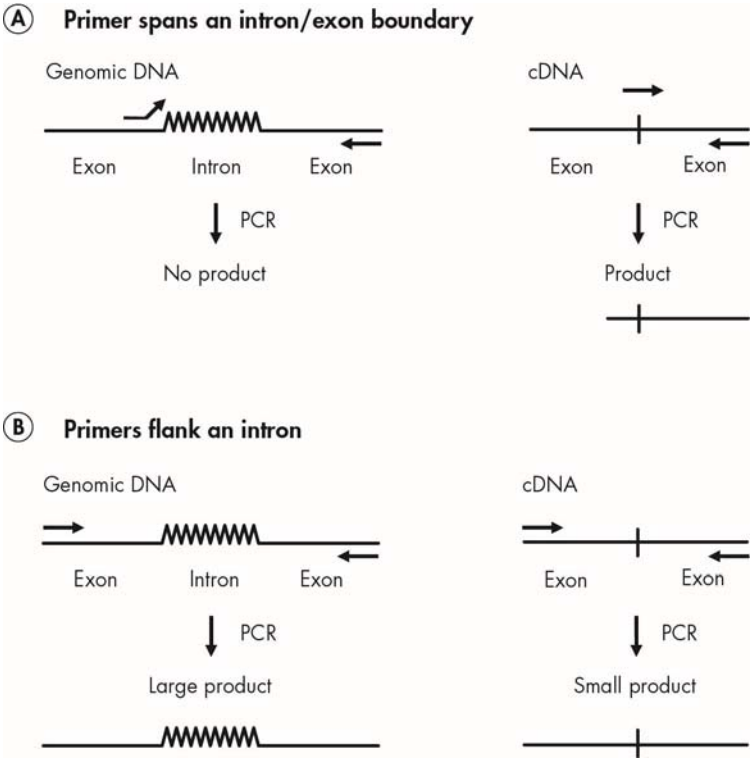

Figure 3. Primer design for PCR. A. Primer design to eliminate or B. detect amplification from contaminating genomic DNA.

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 or when a family of closely related sequences is to be amplified. 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 positions that correspond to the uncertainties in the template sequence.

Hot-start PCR, as enabled by the AllTaq PCR Kits, often improves amplification specificity in PCR using degenerate primers by reducing the formation of nonspecific PCR products and primer–dimers. Table 11 gives recommendations to optimize PCR using degenerate primers. To help determine the best location for degenerate primers, Table 12 lists the codon redundancy of each amino acid.

Table 11. Guidelines for design and use of degenerate primers

<table>
<tr><th></th><th>Description</th></tr>
<tr><td>Sequence</td><td>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.</td></tr>
<tr><td>PCR conditions</td><td>When optimizing two-step PCR using degenerate primers, modify PCR conditions in the following order.</td></tr>
<tr><td>Primer concentration</td><td>First try a primer concentration of 0.25 µM. If this primer concentration results in poor PCR amplification, increase the primer concentration in increments of 0.2 µM until satisfactory results are obtained.</td></tr>
<tr><td>Template concentration</td><td>Increase starting template amount (up to 1 µg).</td></tr>
<tr><td>PCR annealing temperature</td><td>Reduce annealing temperature in steps of 2°C.</td></tr>
<tr><td>Concentration</td><td>● Spectrophotometric conversion for primers: 1 A_{260} unit ≡ 20–30 µg/ml.
● Molar conversions:
<table>
<tr><th>Primer length</th><th>pmol/µg</th><th>12.5 pmol (0.25 µM in 25 µl)</th><th>Primer length (0.25 µM in 20 µl)</th></tr>
<tr><td>18mer</td><td>168</td><td>37 ng</td><td>30 ng</td></tr>
<tr><td>20mer</td><td>152</td><td>42 ng</td><td>34 ng</td></tr>
<tr><td>25mer</td><td>121</td><td>52 ng</td><td>41 ng</td></tr>
<tr><td>30mer</td><td>101</td><td>62 ng</td><td>50 ng</td></tr>
</table>
● Use 0.25–1.0 µM of each primer in PCR. For most applications, a primer concentration of 0.25 µM will be optimal.</td></tr>
</table>

Table 12. 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 E: Sensitive PCR Assays

PCR can be performed to amplify and detect just a single copy of a nucleic acid sequence. However, amplification of such a low number of target sequences is often limited by the generation of nonspecific PCR products and primer–dimers. The combination of AllTaq DNA Polymerase and PCR Buffer increases specificity both at the start of and during PCR. Thus, AllTaq DNA Polymerase is well suited to such challenging and highly sensitive PCR assays.

Nested PCR

If PCR sensitivity is too low, a nested PCR method can increase PCR product yield. Nested PCR involves reverse transcription followed by two rounds of amplification reactions. The first-round PCR is performed according to the AllTaq PCR Protocol. Subsequently, an aliquot of the first-round PCR product, for example 1 µl of a 1:1000 – 1:10000 dilution, is subjected to a second round of PCR. The second-round PCR is performed using two new primers that hybridize to sequences internal to the first-round primer target sequences. In this way, only specific first-round PCR products (and not nonspecific products) will be amplified in the second round. Alternatively, it is possible to use one internal and one first-round primer in the second PCR; this method is referred to as semi-nested PCR.

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 PCR 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 per 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, 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, enzymes, salts, mineral oil and possibly nonspecific amplification products. Before the specific PCR product can be used in subsequent experiments, it is often necessary to remove these contaminants. The QIAquick® and MinElute® systems offer a quick and easy method for purifying the final PCR product (see Ordering Information, page 36). For more information about QIAquick or MinElute products, please visit **www.qiagen.com**.

Appendix G: Control of Contamination

General control of nucleic acid contamination

It is extremely important to include at least one negative control in every round of PCR. This control lacks the template nucleic acid in order to detect possible contamination of the reaction components.

General physical precautions

Separate the working areas for setting up PCR amplifications and RNA 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. Use of freshly distilled water is strongly recommended.

In case of contamination, laboratory benches, apparatus and pipets can be decontaminated by cleaning them with 10% (v/v) 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 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.

Ordering Information

Product	Contents	Cat. no.
AllTaq PCR Core Kit (250)	250 units Taq polymerase: 1 x 50 µl AllTaq Polymerase (5U/µl), 1 x 1.2 ml AllTaq PCR Buffer (5x), 1 x 55 µl dNTP Mix (10 mM/each), 1 x 200 µl Template Tracer (25x), 1 x 50 µl Master Mix Tracer (125x), 1 x 2 ml Q-Solution (5x), 1 x 1.2 ml $MgCl_2$ (25 mM), 1 x 1.9 ml RNase-Free Water	203123
AllTaq PCR Core Kit (1000)	1000 units Taq polymerase: 1 x 200 µl AllTaq Polymerase (5U/µl), 2 x 1.2 ml AllTaq PCR Buffer (5x), 1 x 200 µl dNTP Mix (10mM/each), 1 x 200 µl Template Tracer (25x), 2 x 50 µl Master Mix Tracer (125x), 1 x 2 ml Q-Solution (5x), 1 x 1.2 ml $MgCl_2$ (25 mM), 4 x 1.9 ml RNase-Free Water	203125
AllTaq PCR Core Kit (5000)	5000 units Taq polymerase: 5 x 200 µl AllTaq Polymerase (5U/µl), 9 x 1.2 ml AllTaq PCR Buffer (5x), 5 x 200 µl dNTP Mix (10 mM/each), 4 x 200 µl Template Tracer (25x), 9 x 50 µl Master Mix Tracer (125x), 5 x 2 ml Q-Solution (5x), 2 x 1.2 ml $MgCl_2$ (25 mM), 16 x 1.9 ml RNase-Free Water	203127

Product	Contents	Cat. no.
AllTaq Master Mix Kit (2500)	For 2500 x 20 µl PCR reactions: 10 x 1.25 ml AllTaq Master Mix (4x), 5 x 200 µl Template Tracer (25x), 10 x 50 µl Master Mix Tracer (125x), 20 x 1.9 ml RNase-Free Water	203146
Related Products		
Omniscript Reverse Transcriptase Kits – for reverse transcription using		
Omniscript Reverse Transcriptase Kit (10)	For 10 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	205110
Sensiscript Reverse Transcriptase Kits – for reverse transcription using <50 ng RNA		
Sensiscript Reverse Transcriptase Kit (50)	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix, RNase-Free Water	20521
QIAquick PCR Purification Kit – for direct purification of PCR fragments		
QIAquick PCR Purification Kit (50)*	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
MinElute PCR Purification Kit – for purification of PCR products (70 bp – 4 kb) in low elution volumes		
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
QIAgility® System HEPA/UV (incl. PC)	Robotic workstation for automated PCR setup (with UV light and HEPA filter), notebook computer and QIAgility Software: includes installation and training, 1-year warranty on parts and labor	9001532

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Visit **www.qiagen.com/geneXpression** to find out more about standardized solutions for gene expression analysis – from RNA preparation to real-time RT-PCR.

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