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July 2019

# UCP SYBR<sup>®</sup> Green PCR Handbook

Ultra-clean production (UCP) Master Mix kit  
for real-time PCR and 2-step RT-PCR using  
SYBR<sup>®</sup> Green

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# Kit Contents

<b>UCP SYBR® Green PCR Kit</b>	<b>(100)</b>	<b>(500)</b>
<b>Catalog no.</b>	<b>208012</b>	<b>208014</b>
<b>Number of reactions (20 µl/10 µl)</b>	<b>100/200</b>	<b>500/1000</b>
2x UCP SYBR® Green PCR Master Mix, which contains:	1 ml	5 x 1 ml
<ul style="list-style-type: none"> <li>• UCP DNA Polymerase (comprising <i>Taq</i> DNA Polymerase, Antibody, and Guard)</li> <li>• UCP SYBR® Green PCR Buffer</li> <li>• dNTP mix (dATP, dCTP, dGTP, and dTTP)</li> </ul>		
UCP Yellow Template Dilution Buffer	200 µl	200 µl
UCP ROX™ Reference Dye	250 µl	2 x 250 µl
UCP Water	1.9 ml	4 x 1.9 ml
Quick-Start Protocol	1	1

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## Storage

The UCP SYBR® Green PCR Kit is shipped on dry ice. Store immediately upon receipt at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer and protect from light. When stored under these conditions and handled correctly, kit performance is guaranteed until the expiration date printed on the kit label. UCP SYBR® Green PCR Master Mix, UCP Yellow Template Dilution Buffer, and UCP ROX Reference Dye should be stored protected from light and can also be stored at  $2$ – $8^{\circ}\text{C}$  for up to 6 months or the expiration date printed on the kit label. Ultra-clean production (UCP) reagents are depleted of nucleic acids, and appropriate measures should be taken to prevent any contamination during storage or use.

If desired, UCP ROX Reference Dye can be added to 2x UCP SYBR® Green PCR Master Mix for long-term storage. For details, see “Adding ROX dye to the Master Mix”.

## Intended Use

The UCP SYBR® Green PCR Kit is 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.

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# 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](http://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 UCP SYBR® Green PCR Kit is tested against predetermined specifications to ensure consistent product quality.

# Product Specifications

Component	Description
UCP DNA Polymerase	A modified form of a recombinant 94 kDa DNA polymerase, originally isolated from <i>Thermus aquaticus</i> . UCP DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 2 min 95°C incubation step.
UCP SYBR® Green PCR Buffer	Contains Tris, NH <sup>4+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , and additives enabling fast cycling
dNTP mix	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
Fluorescent dye	SYBR® Green I
UCP ROX Reference Dye	Optimized concentration of fluorescent dye for normalization of fluorescent signals on all instruments from Applied Biosystems®; depleted of potentially contaminating DNA and filled under UCP standards
UCP Yellow Template Dilution Buffer	Ultrapure quality, PCR-grade product depleted of potentially contaminating DNA, and filled under UCP standards
UCP Water	Ultrapure quality, PCR-grade product depleted of potentially contaminating DNA, and filled under UCP standards

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# Introduction

The UCP SYBR® Green PCR Kit provides highly sensitive and rapid real-time quantification of DNA and cDNA targets in an easy-to-use format. The kit can be used in real-time PCR of genomic DNA targets and also in real-time, 2-step RT-PCR of RNA targets following reverse transcription with, for example, the QuantiNova® Reverse Transcription Kit (see “Ordering Information”). The fluorescent dye SYBR® Green I in the Master Mix enables analysis of many different targets without having to synthesize target-specific labeled probes. High specificity and sensitivity in real-time PCR are achieved with the use of a novel hot-start enzyme, UCP DNA Polymerase, together with a specialized real-time PCR buffer based on QIAGEN’s proprietary PCR buffer technology. The Guard molecule, a novel additive, further improves the stringency of the antibody-mediated hot start. The kit also includes a built-in control for visual identification of correct template addition. Additives in the PCR buffer ensure short cycling steps without loss of PCR sensitivity and efficiency.

In addition, the UCP SYBR® Green PCR Kit is engineered to show high inhibitor resistance against substances commonly found in sample types used in microbiome analysis workflows. Dedicated processes are implemented to enable ultra-clean production of this kit; these include depletion of potential DNA background to a minimum, improving data due to removal of bioburden, which could introduce bias in microbial detection workflows.

The UCP SYBR® Green PCR Kit has been optimized for use with all currently available real-time cyclers. The UCP ROX Reference Dye is provided in a separate tube and can be added when using a cycler that requires ROX as a passive reference dye.

## Principle and procedures

### 2x UCP SYBR® Green PCR Master Mix

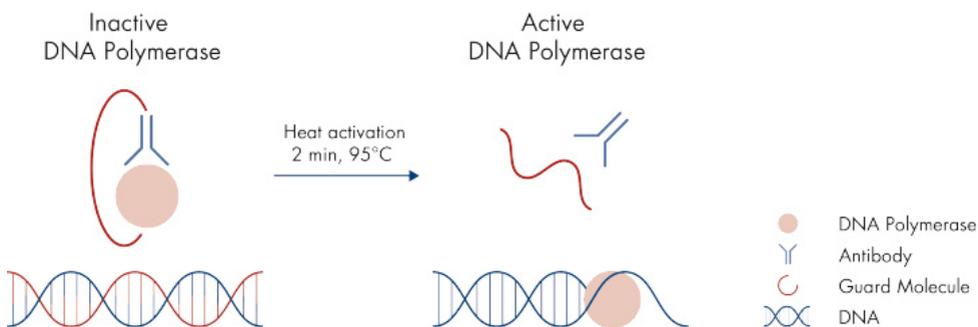
The components of 2x UCP SYBR® Green PCR Master Mix include DNA Polymerase and SYBR® Green PCR Buffer. The optimized Master Mix ensures ultrafast real-time PCR

amplification with high specificity and sensitivity. The SYBR® Green PCR Buffer is also based on the unique QIAGEN PCR buffer system. The buffer contains a balanced combination of KCl and NH<sub>4</sub>Cl, which promotes a high ratio of specific to nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity. Primer annealing is only marginally influenced by the Mg<sup>2+</sup> concentration, so optimization by titration of Mg<sup>2+</sup> is not required.

### Antibody-mediated hot-start mechanism

DNA polymerase is provided in an inactive state that prevents the formation and extension of nonspecifically annealed primers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate quantification. At low temperatures, the UCP DNA Polymerase is kept in an inactive state by the UCP Antibody and a novel additive, UCP Guard, which stabilizes the complex. This improves the stringency of the hot start.

Within 2 min of raising the temperature to 95°C, UCP Antibody and UCP Guard are denatured and UCP DNA Polymerase is activated, enabling PCR amplification (Figure 1). The hot start allows reactions to be set up rapidly and conveniently at room temperature (15–25°C).



**Figure 1. Principle of the hot-start mechanism.** The DNA polymerase is kept in an inactive state by the Antibody and Guard Molecule until the initial heat activation step.

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## Built-in visual control for correct pipetting

The Master Mix supplied with the UCP SYBR® Green PCR Kit contains an inert blue dye that does not interfere with the real-time PCR but increases visibility in the tube or well. UCP Yellow Template Dilution Buffer contains an inert yellow dye. When the template nucleic acid diluted with the UCP Yellow Template Dilution Buffer is added to the Master Mix, the color of the solution changes from blue to green, providing a visual indication of correct pipetting. The use of the UCP Yellow Template Dilution buffer is optional.

## Passive reference dye

For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR-related variations in fluorescence detection. Fluorescence from ROX dye does not change during the course of real-time PCR but provides a stable baseline to which PCR-related fluorescent signals are normalized. Thus, ROX dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or to differences in well position. ROX dye does not interfere with real-time PCR because it is not involved in the reaction and has an emission spectrum that is different from the emission spectrum of SYBR® Green I.

The use of ROX dye is necessary for instruments from Applied Biosystems. The UCP SYBR® Green PCR Kit is provided with a separate tube of UCP ROX Reference Dye. It can be added to the real-time PCR if using a real-time cycler that uses ROX as a passive reference dye. The UCP ROX Reference Dye should be diluted 1:10 in the 1x real-time PCR sample when used on instruments requiring a high ROX concentration and 1:200 for those instruments requiring a low ROX concentration. Refer to Table 1 for details on real-time cyclers that require low or high ROX concentrations.

**Table 1. Real-time cyclers requiring high/low concentrations of ROX dye**

<b>High ROX concentration (1:20 dilution of QN ROX Reference Dye in 1x reaction)</b>	<b>Low ROX concentration (1:200 dilution of QN ROX Reference Dye in 1x reaction)</b>
ABI PRISM® 7000	Applied Biosystems 7500
Applied Biosystems 7300	Applied Biosystems ViiA®7
Applied Biosystems 7900	Applied Biosystems QuantStudio® Systems
Applied Biosystems StepOne®	
Applied Biosystems StepOne Plus	

If required, UCP ROX Reference Dye can be added to 2x UCP SYBR® Green PCR Master Mix for long-term storage (Table 2). For details, see “Adding ROX dye to the Master Mix”.

### Adding ROX dye to the Master Mix

If only Applied Biosystems cyclers will be used with the UCP SYBR® Green PCR Kit, UCP ROX Reference Dye Solution can be added to the 2x UCP SYBR® Green PCR Master Mix for long-term storage, if desired. For information on the concentration of ROX required for Applied Biosystems instruments, refer to Table 1. For reaction setup with a Master Mix that already contains a high concentration of added UCP ROX Reference Dye, refer to Appendix A.

**Table 2. Addition of UCP ROX Reference Dye to the Master Mix**

<b>Volume of 2x UCP SYBR® Green PCR Master Mix (without UCP ROX Reference Dye)</b>	<b>Volume of UCP ROX Reference Dye for high ROX concentration/low ROX concentration</b>
1 ml	100/10 µl

### Special recommendations for microbiome analysis

Preventing unwanted DNA contamination is a key challenge during the analysis of low biomass microbial targets. We suggest setting up PCR reactions in a clean PCR cabinet. All surfaces should be UV decontaminated prior to working. All accessories used (e.g., pipettes, racks, and PCR disposables) should be dedicated for this particular use and should remain in the PCR cabinet. Please refer to standard publications for further recommendations.

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In addition, we recommend including no-template controls (NTCs) and using as much template input and as few PCR cycles as possible. This will help prevent PCR bias. However, in case of small sample amounts or low DNA content, cycle numbers may be increased up to 45 because the background signal from the UCP Master Mix is exceptionally low.

# Protocol: Real-Time PCR and 2-Step RT-PCR

## Important points before starting

- Always start with the cycling conditions specified in this protocol, even if using previously established primer systems. The UCP SYBR® Green PCR Kit has been developed for use in bacterial quantification reactions as well as in 2-step cycling protocols, with a denaturation step at 95°C and a combined annealing/extension step at 60°C. This protocol will also work for primers with a  $T_m$  well below 60°C; however, in case the primer  $T_m$ s are significantly below 60°C (e.g., using the Earth Microbiome Project 16S Primer), the use of a 3-step protocol might be beneficial.
- We recommend using 1 µl of 20x primer mix per 20 µl reaction.
- For the highest efficiency in real-time PCR using SYBR® Green, targets should ideally be 60–200 bp in length. Longer amplicons will also work but need an adaptation of the annealing and extension time.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the DNA polymerase in UCP Master Mix.
- For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.
- The dye in UCP Yellow Template Dilution Buffer allows tracking of pipetted samples in the qPCR. When added to the blue UCP SYBR® Green PCR Master Mix, the color changes from blue to green, indicating the successful addition of the template. The use of this buffer is optional. It is provided as 100x concentrate and should be diluted (using UCP Water) to obtain a final concentration of 1x within samples.\* To generate a template dilution series (e.g., for absolute quantification or determination of PCR efficiency), dilute the 100x concentrate (using UCP Water) to obtain a final concentration

\* Example: Add 0.5 µl Yellow Template Dilution Buffer to a 50 µl sample, which can be used as template in various PCR runs, regardless of the volume added to each reaction. Yellow Template Dilution Buffer can be prediluted using UCP Water. In this example, add 5 µl of 1:10 prediluted Yellow Template Dilution Buffer.

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of 1x UCP Yellow Template Dilution Buffer. The buffer does not affect the sample stability or qPCR.

- This protocol is optimized for quantification of gDNA or cDNA targets using SYBR® Green I with any real-time cycler and condition for fluorescence normalization. The amount of required ROX dye varies, depending on the instrument used:
  - **No requirement for ROX dye:** Rotor-Gene®, Bio-Rad® CFX, Roche® LightCycler® 480, and Agilent® Technologies Mx instruments
  - **Low concentration of ROX dye:** Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems
  - **High concentration of ROX dye:** ABI PRISM 7000, Applied Biosystems 7000, 7300, 7900, and StepOne Real-Time PCR Systems
- Use appropriate techniques to avoid contamination of the ultra-clean reagents with nucleic acids, because this affects background signals in the nontemplate controls. Furthermore, primers should be designed to show no primer-dimer formation, because this might also result in signals in the nontemplate controls.

## Procedure

1. Thaw the 2x UCP SYBR® Green PCR Master Mix, UCP Yellow Template Dilution Buffer, template gDNA or cDNA, primers, UCP ROX Reference Dye (if required), and UCP Water. Mix the individual solutions.
2. Prepare a reaction mix according to Table 3.

Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler.

**Table 3. Reaction setup for UCP SYBR® Green PCR Master Mix Kit**

Component	Volume/reaction		
	96-well block	384-well block	Final concentration
2x UCP SYBR® Green PCR Master Mix	10 µl	5 µl	1x
UCP ROX Reference Dye (Applied Biosystems cyclers only)	1 µl/0.1 µl*	0.5 µl/0.05 µl*	1x
20x primer mix†	0.5–1.4 µl	0.25–0.7 µl	0.25–0.7 µM
UCP Water	Variable	Variable	
Template DNA or cDNA (added at step 4)	Variable	Variable	0.01 pg – 100 ng/reaction
<b>Total reaction volume</b>	<b>20 µl</b>	<b>10 µl</b>	

\* To be used as a 20x concentrate for high ROX cyclers (i.e., ABI PRISM 7000, Applied Biosystems 7300, 7900, and StepOne Real-Time PCR Systems) and as a 200x concentrate for low ROX dye cyclers (i.e., Applied Biosystems 7500, ViiA 7, and QuantStudio Real-Time PCR Systems).

† A 20x primer-probe mix consists of 5 µM forward primer and 5 µM reverse primer in either TE buffer or UCP Water, for a final concentration of 0.25 µM. Primers can either be premixed and added simultaneously or added separately. The primer mix volume needs to be adjusted to achieve the desired final concentration.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR vessels or plates.

4. Add template gDNA or cDNA (10 fg – 100 ng/reaction, depending on target abundance and sample type) to the individual PCR vessels or wells containing the reaction mix.

For 2-step RT-PCR, the volume of the cDNA added (from the undiluted RT reaction) should not exceed 10% of the final PCR volume.

5. Program your real-time cycler according to the manufacturer's instructions using the conditions listed in either Table 4 or Table 5.

Data acquisition should be performed during the combined annealing/extension step.

**Table 4. Real-time cycler conditions for 2-step cycling**

Step	Time	Temperature	Ramp rate	Additional comments
<b>PCR initial activation step</b>	2 min	95°C	Maximal/ fast mode	UCP DNA Polymerase is activated by this heating step
<b>2-step cycling</b>				
Denaturation	10 s	95°C	Maximal/ fast mode	
Combined annealing/ extension	10 s*	60°C	Maximal/ fast mode	Perform fluorescence data collection
Number of cycles	35–45			The number of cycles depends on the amount of template DNA
<b>Melting curve analysis†</b>				

\* If your cycler does not accept this short time for data acquisition, use the shortest acceptable time.

† Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow the instructions provided by the supplier.

For primer sets that do not have an appropriate annealing temperature, or for degenerated or long-fragment assays, we recommend using the 3-step cycling outlined in Table 5.

**Table 5. Real-time cycler conditions for 3-step cycling, (e.g., for Earth Microbiome primer)**

Step	Time	Temperature	Ramp rate	Additional comments
<b>PCR initial activation step</b>	2 min	95°C	Maximal/ fast mode	UCP DNA Polymerase is activated by this heating step
<b>3-step cycling</b>				
Denaturation	10 s	95°C	Maximal/ fast mode	
Annealing	20 s	50–60°C	Maximal/ fast mode	If using degenerated primers or other reasons requiring lower annealing temp
Extension	30 s	72	Maximal/ fast mode	Perform fluorescence data collection
<b>Number of cycles</b>	35–45			The number of cycles depends on the amount of template DNA
<b>Melting curve analysis*</b>				

\* Melting curve analysis is an analysis step built into the software of real-time cyclers. To perform the analysis, follow instructions provided by the supplier.

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6. Place the PCR vessels or plates in the real-time cyclers and start the cycling program.
  7. Perform melting curve analysis of the PCR products.

We strongly recommend routinely performing this analysis, which is built into the software of real-time cyclers, to verify the specificity and identity of PCR products.

**Optional:** Check the specificity of PCR products by agarose gel electrophoresis.

# 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](http://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 or protocols in this handbook or sample and assay technologies (for contact information, visit [www.qiagen.com](http://www.qiagen.com)).

## Comments and suggestions

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### No signal or one or more signals detected late in PCR

- |   |   |
|---|---|
| a) Incorrect cycling conditions             | Always start with the optimized cycling conditions specified in the protocols. Ensure that the cycling conditions include the initial step for activation of UCP DNA Polymerase (95°C for 2 min) and the specified times for denaturation and annealing/extension.                                  |
| b) UCP DNA Polymerase not activated         | Ensure that the cycling program includes the UCP DNA Polymerase activation step (2 min at 95°C) as described in the protocol.   |
| c) Pipetting error or missing reagent       | Check the concentrations and storage conditions of the reagents, including primers, and template nucleic acid. See Appendix B for details on evaluating the concentration of primers. Repeat the PCR. Use the provided UCP Yellow Template Dilution Buffer to prevent errors during reaction setup. |
| d) Wrong or no detection step               | Ensure that fluorescence detection takes place during the combined annealing/extension step.  |
| e) Primer concentration not optimal         | Use each primer at an optimal concentration of 0.7 µM. Check the concentrations of primers by spectrophotometry (see Appendix B).   |
| f) Problems with starting template          | Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions.   |
| g) Insufficient amount of starting template | Increase the amount of template if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample.  |
| h) Insufficient number of cycles            | Increase the number of cycles.  |
| i) Reaction volume too high                 | For 96-well block cyclers, we recommend a final reaction volume of 20 µl. For 384-well block cyclers, we recommend a final reaction volume of 10 µl.  |
| j) PCR product too long                     | For optimal results, PCR products should be between 60 and 200 bp.  |

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### Comments and suggestions

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- k) Primer design not optimal      Check for PCR products by melting curve analysis or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B). Alternatively, use QuantiNova LNA PCR Assays, which are predesigned primer sets for real-time RT-PCR.
- l) No detection activated            Check that fluorescence detection was activated in the cycling program.
- m) Primers degraded                Check for possible degradation of primers on a denaturing polyacrylamide gel.

#### Primer-dimers and/or nonspecific PCR products

- a) Mg<sup>2+</sup> concentration adjusted      Do not adjust the Mg<sup>2+</sup> concentration in 2x UCP SYBR® Green PCR Master Mix.
- b) Primer design not optimal      Check for PCR products by melting curve analysis or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines (see Appendix B). Alternatively, use QuantiNova LNA PCR Assays, which are predesigned primer sets for real-time RT-PCR.
- c) PCR product too long            For optimal results, PCR products should be between 60 and 200 bp. PCR products should not exceed 300 bp.
- d) Primers degraded                Check for possible degradation of primers on a denaturing polyacrylamide gel.

#### Increased fluorescence or C<sub>t</sub> value for “No Template” control

- a) Contamination of reagents      Discard all the components of the assay (e.g., Master Mix and primers). Repeat the assay using new components.
- b) Contamination during reaction setup      Take appropriate precautions during reaction setup, such as using aerosol-barrier pipette tips. For applications using generic microbial primers such as 16S assays, make sure that all used disposables are free of bacterial DNA and that there is no contamination during setup.

#### Varying fluorescence intensity

- a) Contamination of real-time cycler      Decontaminate the real-time cycler according to the manufacturer’s instructions.
- b) Real-time cycler no longer calibrated      Recalibrate the real-time cycler according to the manufacturer’s instructions.

#### All cycler systems

Wavy curve at high template amounts for highly expressed targets      In the analysis settings, reduce the number of cycles used for background calculation (if your real-time cycler allows you to do so) or reduce the amount of template.

#### Applied Biosystems instruments only

ΔRn values unexpectedly too high or too low      The concentration of the UCP ROX Reference Dye is wrong. To choose the right ROX concentration for your cycler, refer to Table 1.

## Appendix A: Reaction Setup Using Master Mix Containing a High Concentration of ROX

**Note:** This appendix is only relevant for a reaction setup using a Master Mix containing a high concentration of ROX. When using Master Mix with low concentration of ROX, the volume of ROX added is negligible; follow the standard reaction setup as described in Table 6.

**Table 6. Reaction setup**

Component	Volume/reaction		Final concentration
	96-well block	384-well block	
2x UCP SYBR® Green PCR Master Mix (containing high concentration of ROX)	12 µl	6 µl	1x
20x primer mix*	0.5–1.4 µl	0.25–0.7 µl	0.25–0.7 µM
UCP Water	Variable	Variable	
Template DNA or cDNA (added at step 4)	Variable	Variable	0.01 pg – 100 ng/reaction
<b>Total reaction volume</b>	<b>20 µl</b>	<b>10 µl</b>	

\* A 20x primer-probe mix consists of 5 µM forward primer and 5 µM reverse primer in either TE buffer or UCP Water, for a final concentration of 0.25 µM. Primers can either be premixed and added simultaneously or added separately. The primer mix volume needs to be adjusted to achieve the desired final concentration.

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# Appendix B: Controlling Contamination

## General control of nucleic acid contamination

It is extremely important to include at least 1 negative control in every round of PCR. This control contains no added template nucleic acid; thus, it is possible to detect contamination of the reaction components.

## General physical precautions

Separate the working areas for setting up PCR amplifications and for 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 pipettes for the PCR Master Mix. The use of pipette tips with hydrophobic filters is strongly recommended.

Prepare and freeze small aliquots of primer solutions. The use of DNA-free water is strongly recommended.

In case of contamination, laboratory benches, apparatuses, and pipettes can be decontaminated by cleaning them with 10% (v/v) commercial bleach solution. Afterwards, the benches and pipettes should be rinsed with distilled water.

For 16S or 18S PCR amplification reactions, we recommend setting up in UV cabinets. All surfaces should be UV decontaminated prior to working. All used accessories – for instance, pipettes, racks, and PCR disposables – should be dedicated to this particular use and should remain in the PCR cabinet. Please refer to standard publications for further recommendations.

## General chemical precautions

PCR stock solutions can also be decontaminated using UV light. However, 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.
UCP SYBR® Green PCR Kit (100)	For 100 x 20 µl reactions: 1 ml 2x UCP SYBR® Green PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 250 µl UCP ROX Reference Dye, 1.9 ml RNase-free water	208012
UCP SYBR® Green PCR Kit (500)	For 500 x 20 µl reactions: 5 x 1 ml 2x UCP SYBR® Green PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 0.5 ml UCP ROX Reference Dye, 4x 1.9 ml RNase-free water	208014
UCP SYBR® Green 16S Quant Kit (100)	For 100 x 20 µl reactions: 1x Microbial DNA Standard, 1x 16S DNA SYBR® Green Assay, 1 ml 2x UCP SYBR® Green PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 250 µl UCP ROX Reference Dye, 1.9 ml RNase-free water	208082
<b>UCP Probe PCR Kit – for highly sensitive, specific, and ultrafast probe-based real-time PCR</b>		
UCP Probe PCR Kit (100)	For 100 x 20 µl reactions: 1 ml 2x UCP Probe PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 250 µl UCP ROX Reference Dye, 4x 1.9 ml UCP Water	208212

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
UCP Probe PCR Kit (500)	For 500 x 20 µl reactions: 5 x 1 ml 2x UCP Probe PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 0.5 ml UCP ROX Reference Dye, 1.9 ml UCP Water	208214
UCP Probe 16S/18S Quant Kit (100)	For 100 x 20 µl reactions: 1x Microbial DNA Standard, 1x 16S/18S DNA Probe Assay, 1 ml 2x UCP Probe PCR Master Mix, 200 µl UCP Yellow Template Dilution Buffer, 250 µl UCP ROX Reference Dye, 4x 1.9 ml UCP Water	208282
<b>Related products</b>		
UCP HiFidelity PCR Kit (100)	For 100 x 25 µl PCR reactions: UCP Master Mix for high-fidelity hot-start PCR and microbiome applications	202742
UCP HiFidelity PCR Kit (500)	For 500 x 25 µl PCR reactions: UCP Master Mix for high-fidelity hot-start PCR and microbiome applications	202744
UCP Multiplex PCR Kit (100)	For 100 x 20 µl PCR reactions: UCP Master Mix for multiplex hot-start PCR and microbiome applications	206742
UCP Multiplex PCR Kit (500)	For 500 x 20 µl PCR reactions: UCP Master Mix for multiplex hot-start PCR and microbiome applications	206744
DNeasy® PowerSoil® Pro Kit (50)	For 50 preps: isolation of microbial genomic DNA from all soil types	47014
DNeasy PowerSoil Pro Kit (250)	For 250 preps: isolation of microbial genomic DNA from all soil types	47016

<b>Product</b>	<b>Contents</b>	<b>Cat. no.</b>
QIAamp® UCP DNA Micro Kit (50)	For 50 preps: ultra-clean DNA purification from small volume sample volumes	56204
QIAamp UCP Pathogen Mini Kit (50)	For 50 preps: microbial DNA purification from whole blood, swabs, cultures, and body fluids	50214
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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

Date	Changes
07/2019	Initial release

## Limited License Agreement for UCP SYBR® Green PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at [www.qiagen.com](http://www.qiagen.com). Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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