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Certal® Residual DNA Detection Handbook

For sensitive real-time PCR detection of residual host cell DNA and an internal control using sequence-specific probes

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Contents

Kit Contents

Certal Residual DNA Detection Kit	**(100)**
Catalog no.	**varies**
Number of 25 µl reactions	**100**
Box 1	
50x Certal Residual DNA Assay	1 vial
50x Certal Internal Control Assay	1 vial
Certal Internal Control DNA	1 vial
Certal Residual DNA Positive Control	220 µl
QuantiTect® Nucleic Acid Dilution Buffer	2 x 1.5 ml
Box 2	
2x Certal ResDNA PCR Master Mix*	1.25 ml
50x ROX™ Dye Solution	210 µl
50x High-ROX Dye Solution	210 µl
UNG (1 U/µl)	100 µl
RNase-Free Water	1.9 ml
Buffer TE	1.9 ml
QuickStart Protocol leaflet	1

* Contains HotStarTaq® DNA Polymerase, Certal ResDNA PCR Buffer, and dNTP mix (dATP, dCTP, dGTP, dTTP, dUTP).

Symbols

Refer to information provided on page 5 of the handbook

Shipping and Storage

Certal Residual DNA Detection Kits are shipped on dry ice. The kits should be stored immediately upon receipt at –15 to –30°C in a constant-temperature freezer and protected from light. When stored under these conditions and handled correctly, the kits can kept until the expiration date on the kit box without showing any reduction in performance.

The 2x Certal ResDNA PCR Master Mix can also be stored at 2–8°C for up to 6 months without showing any reduction in performance.

The Certal Residual DNA Assay and the Certal Internal Control Assay should be stored at –20°C, either lyophilized or reconstituted. Avoid repeated (>6 times) freeze–thaw cycles. The Certal Residual DNA Positive Control should be stored at –20°C and the Certal Internal Control DNA should be stored at –20°C, either lyophilized or reconstituted.

Reconstitute the Certal Internal Control DNA soon after receipt. Avoid repeated (>6 times) freeze–thaw cycles.

Intended Use

The Certal Residual DNA Detection 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.

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

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Certal Residual DNA Detection Kit is tested against predetermined specifications to ensure consistent product quality.

Product Description

Component	Description
50x Certal Residual DNA Assay	Predesigned, optimized, and validated primer–probe set for the detection of residual host cell DNA.
Certal Residual DNA Positive Control	Control to verify the functionality of the residual DNA assay in the case where no residual host cell DNA is detected in the sample.
50x Certal Internal Control Assay	Predesigned, optimized, and validated primer–probe set for the detection of Certal Internal Control DNA; specifically optimized to prevent interference with Certal Residual DNA Assays.
Certal Internal Control DNA	Control to verify the functionality of the real-time PCR itself. Excludes, for example, the presence of PCR inhibitors.
HotStarTaq DNA Polymerase*	A modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. The enzyme is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 15 minute, 95°C incubation step.
Certal ResDNA PCR Buffer*	Optimized buffer for quantitative, duplex, real-time PCR.
dNTP mix*	Contains dATP, dCTP, dGTP, dUTP, and dTTP of ultrapure quality.
50x ROX Dye Solution	Separate tube of passive reference dye for normalization of fluorescent signals on Applied Biosystems® 7500 and ViiA7 Real-Time PCR Systems and, optionally, on Stratagene® instruments from Agilent.
50x High-ROX Dye Solution	Separate tube of passive reference dye for normalization of fluorescent signals on Applied Biosystems 7900 and StepOne™ Real-Time PCR Systems.
Uracil-N-Glycosylase (UNG) (1 U/µl)	Separate tube of pure, nuclease-free recombinant enzyme used to ensure effective elimination of carryover of PCR products.

* Included in 2x Certal ResDNA PCR Master Mix.

Table continued on next page

Product Description, continued

Component	Description
Buffer TE	For reconstitution of lyophilized assays.
QuantiTect Nucleic Acid Dilution Buffer	Proprietary buffer formulation for dilution and storage of nucleic acid standards.
RNase-free water	Ultrapure quality, PCR-grade water.

Introduction

Certal Residual DNA Detection Kits enable sensitive and rapid real-time PCR quantification of gDNA targets using dual-labeled probes (hydrolysis probes, also known as TaqMan® probes) with a variety of real-time PCR cyclers, without requiring optimization. Specific residual host cell DNA will be detected with the Certal Residual DNA Assay, which consists of a premixed primer pair and a hydrolysis probe (FAM™ labeled). The Certal Residual DNA Positive Control is used to verify the functionality of the residual DNA assay in the event that no residual host cell DNA is detectable in the sample.

The Certal Internal Control Assay, containing a premixed primer pair and a hydrolysis probe (MAX labeled), and the Certal Internal Control DNA, are for use as a universal amplification control. The Certal Internal Control is detected in the same tube with the residual DNA target in a duplex PCR reaction to test for successful amplification, and to exclude, for example, the presence of PCR inhibitors.

When working with biopharmaceuticals, it is essential to evaluate the final therapeutic material to ensure that it is free of contaminating substances, such as residual DNA from the host cell. Quantitative data is needed to ensure a safe, high-quality therapeutic product. QIAGEN's integrated system for molecular testing — the QIAsymphony® RGQ — delivers optimized and ready-to-use solutions for automated purification and quantification of residual host cell DNA and viral nucleic acids. This includes sample preparation on the QIAsymphony SP using QIAsymphony Certal Kits and detection on the Rotor-Gene® Q PCR cycler. This workflow ensures the generation of sensitive and reliable results, regardless of whether the starting material is derived from bioprocess purification buffer, cell culture supernatant samples, or vaccine preparations.

2x Certal ResDNA PCR Master Mix

The 2x Certal ResDNA PCR Master Mix contains HotStarTaq DNA Polymerase and Certal ResDNA PCR Buffer, but no ROX passive reference dye; this is supplied in separate tubes. The optimized master mix ensures that the residual DNA target sequence in a duplex reaction is amplified with the same high PCR efficiency and sensitivity as the target sequence in a corresponding singleplex reaction. This leads to high reproducibility of data with high sensitivity and broad dynamic range of the PCR reaction.

HotStarTaq DNA Polymerase

HotStarTaq DNA Polymerase is a modified form of QIAGEN's *Taq* DNA Polymerase. It is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer-dimers during reaction setup and the first denaturation step.

Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is activated at the start of a reaction by a 15-minute, 95°C incubation step. The hot start enables reactions to be set up rapidly and conveniently at room temperature.

Certal ResDNA PCR Buffer

Certal ResDNA PCR Buffer has been specifically developed for quantitative, duplex, real-time PCR using sequence-specific probes. In addition to various salts and additives, the buffer also contains a specially optimized combination of KCl and $(NH_4)_2SO_4$, 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. The buffer also contains synthetic Factor MP, which supports a reliable duplex PCR. Factor MP increases the local concentration of primers and probes at the DNA template and stabilizes specifically bound primers and probes, allowing efficient annealing and extension. This combination of Certal ResDNA PCR Buffer components enables reliable detection of the genomic DNA-specific target with a broad dynamic range and high sensitivity in the presence of the Certal Internal Control.

Certal Residual DNA Assay

Certal Residual DNA Assays are predesigned assays based on hydrolysis probe detection. Each Certal Residual DNA Assay consists of an optimized primer pair and FAM labeled hydrolysis probe for reliable detection of residual genomic DNA. Human background DNA (30 ng) and Certal Internal Control DNA will not interfere with the Certal Residual DNA Assay.

The Certal Residual DNA Assay is supplied in a lyophilized format and should be dissolved prior to the first use according to Table 4, page 18.

Certal Residual DNA Positive Control

Certal Residual DNA Positive Control provides a positive control for the amplification reaction and enables the correct interpretation of negative detection results.

Certal Internal Control Assay

The Certal Internal Control Assay allows simultaneous amplification of the internal control DNA and the residual DNA in duplex, real-time PCR using sequence-specific probes. The premixed Certal Internal Control Assay contains a forward and reverse primer and a hydrolysis probe for the detection of the internal control DNA, and has been specifically optimized to prevent interference with Certal Residual DNA Assays. The Certal Internal Control Assay employs MAX as a reporter dye. With excitation/emission maxima of

524/557 nm, MAX dye has a spectral profile that enables detection in the yellow channel of Rotor-Gene cyclers. It can also be detected in the same channel as HEX™, JOE™, or VIC®, and therefore can be used with most real-time cyclers.

The Certal Internal Control Assay is supplied in a lyophilized format and should be dissolved prior to the first use according to Table 4, page 18.

Certal Internal Control DNA

Certal Internal Control DNA provides a positive control for the amplification reaction, enabling the user to identify possible PCR inhibition and to correctly interpret negative detection results.

Certal Internal Control DNA is a synthetic DNA construct with a unique and artificial sequence. The sequence targeted by the Certal Internal Control Assay lacks homology with any sequences in the GenBank® database.

Certal Internal Control DNA is supplied lyophilized and should be dissolved prior to first use according to Table 4, page 18.

ROX passive reference dye

Master mix supplied with the Certal Residual DNA Detection Kit does not contain ROX dye and can be used directly with all instruments that do not require ROX dye for fluorescence normalization. ROX is not required for Rotor-Gene cyclers, or for instruments from Bio-Rad, Agilent, and Roche.

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. The use of ROX dye is necessary for instruments from Applied Biosystems and is optional for Stratagene instruments from Agilent.

Each Certal Residual DNA Detection Kit also includes two separate vials of ROX dye solutions of different concentrations, which can be added to reactions depending on the real-time cycler used (see Table 1, page 11).

The 50x ROX Dye Solution is intended for use with cyclers that require a lower concentration of ROX dye for fluorescence normalization (e.g., Applied Biosystems 7500 Real-Time PCR System) and for use with cyclers that allow optional use of ROX dye (e.g., Stratagene instruments from Agilent).

The 50x High-ROX Dye Solution is provided at a higher concentration that is optimal for other instruments from Applied Biosystems (models 7000, 7300, 7700, 7900HT, StepOne, and StepOnePlus™). For use with instruments requiring ROX, the user must add the ROX Dye Solution to the master mix during reaction setup.

Table 1. Addition of ROX dye to master mix for long-term storage*

Kit	50x ROX Dye Solution or 50x High-ROX Dye Solution	2x Certal ResDNA PCR Master Mix
Certal Residual DNA Detection Kit (100)	50 µl	1.25 ml

* If ROX dye is premixed with the 2x Certal ResDNA PCR Master Mix as indicated above, the premixed master mix has a 1.92x concentration. Adjust the volume of master mix to be added to the reaction accordingly (add 13 µl premixed master mix to each 25 µl reaction).

QuantiTect Nucleic Acid Dilution Buffer

QuantiTect Nucleic Acid Dilution Buffer is intended for dilution of nucleic acids that are used to generate standard curves or are used as positive controls in real-time PCR. The buffer stabilizes RNA and DNA standards during dilution and reaction setup and prevents loss of nucleic acids on plastic surfaces, such as tubes or pipet tips. QuantiTect Nucleic Acid Dilution Buffer is ready to use and is free of RNases and DNases. Proper use of QuantiTect Nucleic Acid Dilution Buffer enables safe and accurate dilution of small amounts of nucleic acids typically used as standards for analysis of pathogen nucleic acids.

Aliquots of diluted standards can be stored in QuantiTect Nucleic Acid Dilution Buffer at –15 to –30°C for up to 6 months. Avoid repeated freezing and thawing.

QuantiTect Nucleic Acid Dilution Buffer is also intended for reconstitution of the Certal Internal Control DNA (see Table 4, page 18).

Use of uracil-N-glycosylase

The Certal Residual DNA Detection PCR Kit contains dUTP, which partially replaces dTTP, enabling the optional use of a uracil-N-glycosylase (UNG) pretreatment of the reaction if contamination with carryover of PCR products is suspected.

UNG can be added to the PCR at an appropriate concentration (see Table 6, page 21; Table 9, page 24; Table 12, page 28). An incubation step of 50°C for 2 minutes is added to the cycling program, before activation of HotStarTaq DNA Polymerase. During this incubation step, UNG removes uracil from dUMP incorporated into any contaminating molecules, leaving apyrimidinic sites. During the activation step of HotStarTaq DNA Polymerase (15 minutes at 95°C), the UNG is inactivated, and contaminating molecules are destroyed by hydrolysis of the phosphate backbone at the abasic sites. During subsequent cycling, only target nucleic acid will be amplified; contaminating molecules from previous reactions will not be amplified.

Dual-labeled probes

The Certal Residual DNA Detection Kit contains optimized, ready-to-use primer–probe sets. For more details about sequence-specific probes and their handling, see Table 4, page 18.

Hydrolysis probes (e.g., TaqMan probes) included in the Certal Residual DNA Assay and the Certal Internal Control Assay, are sequence-specific oligonucleotides with a fluorophore and a quencher moiety attached (see Figure 1). The fluorophore is at the 5' end of the probe, and the quencher moiety is usually located at the 3' end, or internally. During the extension phase of PCR, the probe is cleaved by the 5'→3' exonuclease activity of *Taq* DNA polymerase, separating the fluorophore and the quencher moiety. This results in detectable fluorescence proportional to the amount of accumulated PCR product.

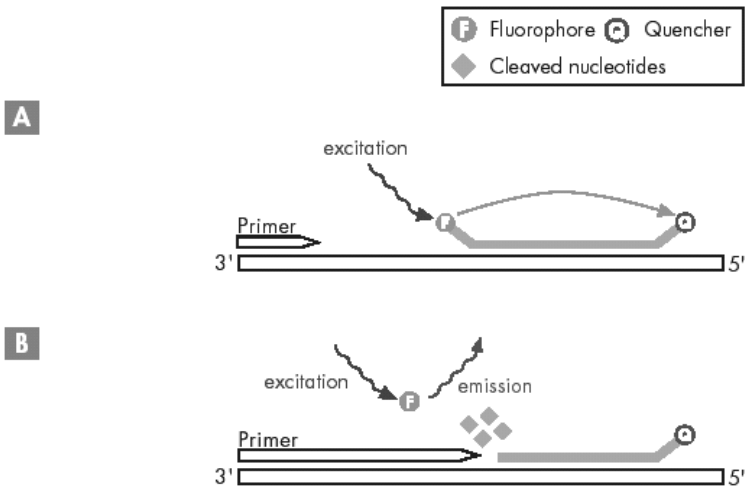


Figure 1. Principle of TaqMan probes in quantitative, real-time PCR. **A** Both the TaqMan probe and the PCR primers anneal to the target sequence during the PCR annealing step. The proximity of the fluorophore with the quencher results in efficient quenching of fluorescence from the fluorophore. **B** During the PCR extension step, *Taq* DNA polymerase extends the primer. When the enzyme reaches the TaqMan probe, its 5'→3' exonuclease activity cleaves the fluorophore from the probe. The fluorescent signal from the free fluorophore is measured. This signal is proportional to the amount of accumulated PCR product.

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.

- Nuclease-free (DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive detection of nucleic acids.
- Cooling device or ice
- Real-time PCR thermal cycler (we recommend the Rotor-Gene Q for high-precision results; for details, visit www.qiagen.com/goto/Rotor-GeneQ)
- PCR tubes, plates (use thin-walled PCR tubes or plates recommended by the manufacturer of your real-time cycler), or Rotor-Discs®
- **Optional**: QIAsymphony SP/AS instruments for fully integrated automation of complete workflows, from sample preparation to assay setup; for details, visit www.qiagen.com/goto/QIAsymphony.

Important Notes

Guidelines for effective duplex detection of residual host cell DNA and the Certal Internal Control

Certal Residual DNA Detection Kits are optimized for use in probe-based, duplex, real-time PCR with the Certal Internal Control. Please read the following guidelines before starting.

- Check the real-time cycler user manual for **correct setup for duplex analysis** (e.g., setting up detection of 2 different dyes from the same well). Be sure to activate the detector for both reporter dyes used. For recommendations on filters, calibration, and ROX dye see Table 3, page 16.
- The Certal Residual DNA Assay uses FAM as a reporter dye. With an excitation/emission maxima of 483/533 nm, FAM dye has a spectral profile allowing detection in the same channel as SYBR® Green dye.
- The Certal Internal Control Assay uses MAX as a reporter dye. With excitation/emission maxima of 524/557 nm, MAX dye has a spectral profile allowing detection in the yellow channel on Rotor-Gene cyclers and same channel as HEX, JOE, or VIC for other cyclers.
- If using MAX dye for the first time on an instrument, ensure that detection is carried out using the correct channel or filter. For details, see the instrument user manual.
- Use the **cycling conditions specified in the protocol** you are following.
- Optimal analysis settings (i.e., baseline settings and threshold values) for both reporter dyes are a prerequisite for accurate detection data. For details, check the literature from the manufacturer of your real-time cycler.
- Include appropriate controls in each real-time PCR run to give additional information for interpretation of results. For details, see "Controls", page 17 and Appendix A, page 34.
- Before starting, check whether the instrument requires the addition of ROX to the duplex reaction as a reference dye. The required ROX concentration may vary between different cyclers. See Table 3, page 16 for recommendations for different instruments.

  Note: If there are no specific recommendations listed for your real-time cycler; refer to the user manual or other technical documentation for the instrument to determine the ROX concentration needed for duplex analysis.

Selecting protocols

To select the correct protocol to use with your real-time cycler, refer to Table 2. In general, the following cyclers are not compatible with duplex, real-time PCR: GeneAmp® 5700, MyiQ™, and DNA Engine Opticon® (i.e., the single-color machine). The protocol is not optimized for use with LightCycler® 1.x and 2.x real-time cyclers.

Table 2. Choosing the correct PCR protocol for your real-time cycler

Cycler	Protocol
Rotor-Gene Q	Protocol 1, page 20
LightCycler 480 (96- and 384-well)	Protocol 2, page 23
ABI PRISM® 7900HT (96- and 384-well), Applied Biosystems 7500 and 7500 Fast, ABI ViiA7	Protocol 3, page 26

Selecting instrument setup

Duplex, real-time PCR requires the simultaneous detection of two different fluorescent reporter dyes. The Certal Internal Control Assay provided with the Certal Residual DNA Detection Kit uses the reporter dye MAX, which has excitation/emission maxima of 524/557 nm, and a nonfluorescent quencher (Iowa Black®). For accurate detection, the fluorescence spectrum of the residual host cell DNA assay dye must be well separated from the MAX spectrum or exhibit only minimal overlap. Please read the general recommendations and instrument-specific recommendations on the following pages before starting.

Note: If there are no specific recommendations below for your real-time cycler, please refer to the user manual or other technical documentation for your instrument to find out which filters can be used for the recommended reporter dyes in duplex analysis.

Table 3. Instrument-specific requirements for filter or channel, calibration, and ROX concentration

Instrument	Filter/channel for detection of residual DNA assay (FAM)	Filter/channel for detection of internal control assay (MAX)	Calibration	ROX Dye Solution*
Rotor-Gene Q[†]	Green channel	Yellow channel	Not required	Not required
Applied Biosystems 7500, ABI ViiA7	FAM/SYBR Green	VIC/JOE	Required for new dyes[‡]	ROX Dye Solution
Applied Biosystems 7900HT, other ABI instruments	FAM/SYBR Green	VIC/JOE	Required for new dyes[‡]	High-ROX Dye Solution
LightCycler 480[§]	Channel 2 FAM (483/533)	Channel 3 HEX (523/568)	Use color compensation file	Not required

* The master mix supplied with the Certal Residual DNA Detection Kit does not contain ROX dye and can be used directly with all instruments that do not require ROX dye for fluorescence normalization. Each Certal Residual DNA Detection Kit also includes two separate vials of ROX dye solutions of different concentrations, which can be added to reactions, depending on the real-time cycler used.

† Refer to the user manual supplied with the Rotor-Gene cycler for additional information on setting up detection channels and correctly setting up the instrument for duplex analysis.

‡ Before using a reporter dye for the first time on the instrument, a pure dye calibration of the real-time cycler must be performed. See the user manual for details on calibration. If the instrument has been calibrated for VIC, this calibration can be used for the detection of MAX. In this case, create a new detector for the detection of the Certal Internal Control Assay ("MAX/IowaBlack") and assign VIC as the reporter dye. For the quencher dye, select "None".

§ It is recommended to use a color compensation file on the LightCycler 480 system that contains information to correct crosstalk between the different detection channels. Refer to the LightCycler 480 user manual for additional information on activating and deactivating detection channels and correctly setting up the instrument for duplex analysis.

Controls

- Ensure that at least one positive control, as well as one negative control (e.g., PCR-grade water), are included per qPCR run.
- For interpretation of PCR results for samples and controls, see Appendix A, page 34.

No template control (NTC)

At least one NTC reaction should be included in each PCR run, containing all the components of the reaction except for the residual genomic DNA/positive control. This enables detection of contamination in the reagents.

Note: The Certal Internal Control DNA will be detected in the NTCs if added to the reaction mix as an amplification control (see "Internal control").

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run that contains a sample known to include the targeted genomic DNA. A positive control serves to prove the functionality of the Certal Residual DNA Probe Assay (e.g., the correct setup of the reaction mix). The Certal Residual DNA Positive Control, or known positive samples can be used as positive controls.

A positive sample is usually a substitute for an absolute standard and is used only to test for presence or absence of the target signal.

Internal control

For increased process safety in residual DNA detection assays, an internal control is detected in the same tube as the genomic DNA target in a duplex PCR. The internal control tests for successful amplification and excludes, for example, the presence of PCR inhibitors. The Certal Residual DNA Detection Kit provides a ready-to-use internal control for universal use with different residual DNA detection assays, without the need for optimization. Each Certal Residual DNA Detection Kit includes the Certal Internal Control Assay and Certal Internal Control DNA for use as an amplification control by direct addition to the reaction mix.

Please note that adding Certal Internal Control DNA to the reaction mix as an amplification control will result in positive internal control signals in NTCs. These signals serve as a reference to assure that the internal control DNA has been successfully amplified. If the internal control signal is detected in the NTCs, but not in sample reactions, this may indicate the presence of inhibitors or other disturbances of the sample reaction.

For guidelines on data interpretation of genomic and internal control detection, see Appendix A, page 34.

Reconstitution and use of assays and control DNA

Reconstitution of components

To reconstitute a tube of Certal Residual DNA Assay or Certal Internal Control Assay, briefly centrifuge the tube, add Buffer TE (provided with the kit), and mix by vortexing the tube 4–6 times (Table 4). Assays should be stored protected from light in sterile, nuclease-free TE buffer in small aliquots at –20°C. Fluorescently labeled probes are usually stable under these conditions for at least 6–9 months. Repeated freeze–thaw cycles should be avoided, since they may lead to degradation.

To reconstitute a tube of Certal Internal Control DNA, briefly centrifuge the tube, add QuantiTect Nucleic Acid Dilution Buffer (supplied) and mix by vortexing the tube 4–5 times. Certal Residual DNA Positive Control and reconstituted Certal Internal Control DNA should be frozen in aliquots to avoid repeated freezing and thawing. Reconstituted Certal Residual DNA Positive Control DNA and Certal Internal Control DNA should be stored at –20°C. Lyophilized Certal Residual DNA Assays are stable for 18 months when stored protected from light at –20°C.

Table 4. Product reconstitution

Component	Volume	Buffer
50x Certal Residual DNA Assay	55 µl	TE
50x Certal Internal Control Assay	55 µl	TE
Certal Internal Control DNA	550 µl	QuantiTect Nucleic Acid Dilution Buffer

Use of Certal Residual DNA Positive Control

After thawing, add the Certal Residual DNA Positive Control solution to the reaction mixture (see Protocols 1–3, pages 20–26).

Successful amplification results in a C_T value of 28 ± 4 for the Certal Residual DNA Positive Control; the deviation is a result of real-time cycler and data analysis variance.

Use of Certal Internal Control DNA

After reconstitution of the lyophilized Certal Internal Control DNA in QuantiTect Nucleic Acid Dilution Buffer, add the resulting Certal Internal Control DNA solution to the reaction mixture (see Protocols, pages 20–26).

Successful amplification results in a C_T value of 28 ± 4 for the Certal Internal Control. The deviation is a result of real-time cycler and data analysis variance.

Use of known genomic DNA

To calculate the absolute amount of residual DNA, perform a 10-fold dilution series of a known concentration of genomic DNA from the respective cell line. It should contain at least 5 data points in the same range that is expected from the unknown sample (e.g., dilution standards should range from 30 ng–30 fg). Because each cell line may differ in terms of (partial) polyploidy and repetitive sequences due to genomic rearrangements, it is recommended to use genomic DNA from the same cell line as the unknown sample to generate the standard curve. For more information, see Appendix B, page 36.

Protocol: Residual DNA Detection on Rotor-Gene Cyclers

Important points before starting

- The 2x Certal ResDNA PCR Master Mix contains dUTP. This allows for a uracil-N-glycosylase (UNG) pretreatment of the reaction if contamination due to carryover of PCR products is suspected.
- The PCR must start with an initial incubation step of 15 minutes at 95°C to activate HotStarTaq DNA Polymerase.
- Be sure to activate the detector for each reporter dye used (target gene [FAM] and internal control [MAX], which can serve as a VIC, JOE, or HEX replacement without the need for an extra calibration step). For details on channels and calibration see Table 3, page 16 and Appendix C.
- Sensitive, automated residual DNA extraction can be performed using the QIAsymphony Certal Extraction Kit on the QIAsymphony SP/AS. The purified eluate is free of contaminating substances, ensuring optimal real-time PCR with the Certal Residual DNA Detection Kit.

Procedure

1. **Thaw 2x Certal ResDNA PCR Master Mix, Certal Residual DNA Assay, Certal Residual DNA Positive Control, Certal Internal Control DNA, Certal Internal Control Assay, Buffer TE, QuantiTect Nucleic Acid Dilution Buffer, and RNase-free water. Thoroughly mix the individual solutions (by pipetting repeatedly up and down or by pulse vortexing), then place on ice.**

   If using for the first time, reconstitute the Certal Residual DNA Assay, Certal Internal Control Assay, and Certal Internal Control DNA according to Table 4, page 18.

2. **Prepare Certal Residual DNA Positive Control mix according to Table 5.**

Table 5. Preparation of Certal Residual DNA Positive Control mix

Component	Volume/reaction (µl)
Certal Residual DNA Positive Control	0.5
Nucleic Acid Dilution Buffer	7

3. **Prepare a reaction mix according to Table 6.**

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep samples, controls, and Certal Internal Control DNA on ice or in a cooling device.

Table 6. Reaction mix setup

Component	Volume/reaction (µl)
2x Certal ResDNA PCR Master Mix	12.5 µl
50x Certal Residual DNA Assay	0.5 µl
50x Certal Internal Control Assay	0.5 µl
Certal Internal Control DNA	0.5 µl
RNase-free water	3.25 µl
Template DNA (e.g., Certal Residual DNA Positive Control mix or sample)	7.5 µl
UNG (1 U/µl) (recommended)	0.25 µl
Total volume/reaction	25 µl

4. **Mix the reaction mix thoroughly (by pipetting up and down or pulse vortexing), then dispense appropriate volumes into PCR tubes or Rotor-Disc wells.**
5. **Add 7.5 µl of the template genomic DNA or positive control mix to the individual PCR tubes or Rotor-Disc wells.**

   Note: Ensure that the reaction mix and template are thoroughly mixed.
6. **Program the Rotor-Gene Q according to Table 7.**

   Data acquisition should be performed during the combined annealing/extension step. Consult the Rotor-Gene user manual for instrument setup for duplex analysis (e.g., setting up detection of multiple dyes from the same well). Be sure to activate the detector for each reporter dye used (target gene [FAM, green channel] and internal control [MAX, yellow channel]. We recommend determining the fluorescence range for your genomic DNA target starting with the default settings (gain 5 for green and yellow).

Table 7. Cycling conditions for the Rotor-Gene Q

Cycles	Temperature	Time	Step	Additional comments
1	50°C	2 min	UNG treatment (recommended)	dUMP-containing PCR products resulting from carryover contamination will be eliminated by UNG
1	95°C	15 min	Initial PCR activation step	HotStarTaq DNA Polymerase is activated by this step
2-step cycling:				Optimal performance is only assured using these cycling conditions
45	94°C	30 sec	Denaturation	
	60°C	1 min	Annealing and extension	Combined annealing and extension step with fluorescence data collection

7. **Place the PCR tubes or Rotor-Disc into the Rotor-Gene Q and start the PCR cycling program.**
8. **Perform data analysis.**

   Before performing data analysis select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification of data.

   The internal control will be always detectable, including in the no template control (NTC) sample.

Protocol: Residual DNA Detection on Cyclers Not Requiring ROX Reference Dye

Important points before starting

- This protocol is optimized for Roche LightCycler 480, and cyclers from Bio-Rad, Cepheid, Eppendorf, and Agilent.
- The 2x Certal ResDNA PCR Master Mix contains dUTP. This allows for a uracil-N-glycosylase (UNG) pretreatment of the reaction if contamination due to carryover of PCR products is suspected.
- The PCR must start with an initial incubation step of 15 minutes at 95°C to activate HotStarTaq DNA Polymerase.
- Be sure to activate the detector for each reporter dye used (target gene [FAM] and internal control [MAX], which can serve as a VIC, JOE, or HEX replacement without the need for an extra calibration step). Depending on the instrument, it may also be necessary to perform a calibration procedure for both reporter dyes before they are used for the first time. For details on channels and calibration see Table 3, page 16 and Appendix C.
- Sensitive, automated residual DNA extraction can be performed using the QIAsymphony Certal Extraction Kit on the QIAsymphony SP/AS. The purified eluate is free of contaminating substances, ensuring optimal real-time PCR with the Certal Residual DNA Detection Kit.

Procedure

1. **Thaw 2x Certal ResDNA PCR Master Mix, Certal Residual DNA Assay, Certal Residual DNA Positive Control, Certal Internal Control DNA, Certal Internal Control Assay, Buffer TE, QuantiTect Nucleic Acid Dilution Buffer, and RNase-free water. Thoroughly mix the individual solutions (by pipetting repeatedly up and down or by pulse vortexing), then place on ice.**

   If using for the first time, reconstitute the Certal Residual DNA Assay, Certal Internal Control Assay, and Certal Internal Control DNA according to Table 4, page 18.

2. **Prepare Certal Residual DNA Positive Control DNA mix according to Table 8.**

Table 8. Preparation of Certal Residual DNA Positive Control mix

Component	Volume/reaction (µl)
Certal Residual DNA Positive Control	0.5
Nucleic Acid Dilution Buffer	7

3. **Prepare a reaction mix according to Table 9.**
   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep samples, controls, and Certal Internal Control DNA on ice or in a cooling device.

Table 9. Reaction mix setup

	Cyclers not requiring ROX	
Component	**96-well**	**384-well**
2x Certal ResDNA PCR Master Mix	12.5 µl	10.0 µl
50x Certal Residual DNA Assay	0.5 µl	0.4 µl
50x Certal Internal Control Assay	0.5 µl	0.4 µl
Certal Internal Control DNA	0.5 µl	0.5 µl
RNase-free water	3.25 µl	1.0 µl
Template DNA (e.g., Certal Residual DNA Positive Control mix or sample)	7.5 µl	7.5 µl
UNG (1 U/µl) (recommended)	0.25 µl	0.2 µl
Total volume/reaction	25 µl	20 µl

4. **Mix the reaction mix thoroughly (by pipetting up and down or pulse vortexing), then dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**
5. **Add 7.5 µl of the template gDNA or Certal Residual DNA Positive Control mix to the individual PCR tubes or wells.**
   Note: Ensure that the reaction mix and template are thoroughly mixed.

6. **Program the real-time cycler according to Table 10.**

   Data acquisition should be performed during the combined annealing/extension step. Consult the real-time cycler manual for proper instrument setup for duplex analysis (e.g., setting up detection of multiple dyes from the same well). Be sure to activate the detector for each reporter dye used (target gene [FAM] and internal control [MAX], which can serve as a VIC, JOE, or HEX replacement without the need for an extra calibration step).

Table 10. Cycling conditions

Cycles	Temperature	Time	Step	Additional comments
1	50°C	2 min	UNG treatment (recommended)	dUMP-containing PCR products resulting from carryover contamination will be eliminated by UNG
1	95°C	15 min	Initial PCR activation step	HotStarTaq DNA Polymerase is activated by this step
2-step cycling:				Optimal performance is only assured using these cycling conditions
45	94°C	1 min	Denaturation	
	60°C	1 min	Annealing and extension	Combined annealing and extension step with fluorescence data collection

7. **Place the PCR tubes in the real-time cycler and start the PCR cycling program.**
8. **Perform data analysis.**

   Before performing data analysis select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification of data.

   The internal control will be always detectable, including in the no template control (NTC) sample.

Protocol: Residual DNA Detection on Cyclers Requiring ROX Reference Dye

Important points before starting

- This protocol is optimized for ABI 7500, ViiA7, ABI 7900, and other ABI instruments requiring ROX as a reference dye.
- The 2x Certal ResDNA PCR Master Mix contains dUTP. This allows for a uracil-N-glycosylase (UNG) pretreatment of the reaction if contamination due to carryover of PCR products is suspected.
- The PCR must start with an initial incubation step of 15 minutes at 95°C to activate HotStarTaq DNA Polymerase.
- When using real-time cyclers from Applied Biosystems, the protocol is performed in the presence of a ROX passive reference dye included in the Certal Residual DNA Detection Kit. Two separate vials of ROX dye solution are included with the Certal Residual DNA Detection Kit. The 50x ROX Dye Solution is intended for use with cyclers that require a lower concentration of ROX dye for fluorescence normalization (e.g., Applied Biosystems 7500 Real-Time PCR Systems) and for use with cyclers that allow optional use of ROX dye (e.g., Stratagene instruments from Agilent). The 50x High-ROX Dye Solution is provided at a higher concentration that is optimal for other instruments from Applied Biosystems (models 7000, 7300, 7700, 7900HT, StepOne, and StepOnePlus).
- Be sure to activate the detector for each reporter dye used (target gene [FAM] and internal control [MAX], which can serve as a VIC, JOE, or HEX replacement without the need for an extra calibration step). Depending on the instrument, it may also be necessary to perform a calibration procedure for both reporter dyes before they are used for the first time. For details on channels and calibration see Table 3, page 16 and Appendix C.
- Sensitive, automated residual DNA extraction can be performed using the QIAsymphony Certal Extraction Kit on the QIAsymphony SP/AS. The purified eluate is free of contaminating substances, ensuring optimal real-time PCR with the Certal Residual DNA Detection Kit.

Procedure

1. **Thaw 2x Certal ResDNA PCR Master Mix, Certal Residual DNA Assay, Certal Residual DNA Positive Control, Certal Internal Control DNA, Certal Internal Control Assay, Buffer TE, QuantiTect Nucleic Acid Dilution Buffer, and RNase-free water. Thoroughly mix the individual solutions (by pipetting repeatedly up and down or by pulse vortexing), then place on ice.**

If using for the first time, reconstitute the Certal Residual DNA Assay, Certal Internal Control Assay, and Certal Internal Control DNA according to Table 4, page 18.

2. **Prepare Certal Residual DNA Positive Control DNA mix according to Table 11.**

Table 11. Preparation of Certal Residual DNA Positive Control mix

Component	Volume/reaction (µl)
Certal Residual DNA Positive Control	0.5
Nucleic Acid Dilution Buffer	7

3. **Prepare a reaction mix for cyclers that require ROX according to Table 12.**

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep samples, controls, and Certal Internal Control DNA on ice or in a cooling device.

Table 12. Reaction mix setup

	ABI 7500/ViiA7	ABI 7900	
Component	**96-well**	**96-well**	**384-well**
2x Certal ResDNA PCR Master Mix	12.5 µl	12.5 µl	10 µl
50x ROX Dye Solution	0.5 µl	–	–
50x High ROX Dye Solution	–	0.5 µl	0.4 µl
50x Certal Residual DNA Assay	0.5 µl	0.5 µl	0.4 µl
50x Certal Internal Control Assay	0.5 µl	0.5 µl	0.4 µl
Certal Internal Control DNA	0.5 µl	0.5 µl	0.5 µl
RNase-free water	2.75 µl	2.75 µl	0.6 µl
Template DNA (e.g., Certal Residual DNA Positive Control mix or sample)	7.5 µl	7.5 µl	7.5 µl
UNG (1 U/µl) (recommended)	0.25 µl	0.25 µl	0.2 µl
Total volume/reaction	25 µl	25 µl	20 µl

4. **Mix the reaction mix thoroughly (by pipetting up and down or pulse vortexing), then dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**
5. **Add 7.5 µl of the template gDNA or Certal Residual DNA Positive Control mix to the individual PCR tubes or wells.**

   Note: Ensure that the reaction mix and template are thoroughly mixed.

6. **Program the real-time cycler according to Table 13.**

   Data acquisition should be performed during the combined annealing/extension step. Consult the real-time cycler manual for proper instrument setup for duplex analysis (e.g., setting up detection of multiple dyes from the same well). Be sure to activate the detector for each reporter dye used (target gene [FAM] and internal control [MAX], which can serve as a VIC or HEX replacement without the need for an extra calibration step).

Table 13. Cycling conditions

Cycles	Temperature	Time	Step	Additional comments
1	50°C	2 min	UNG treatment (recommended)	dUMP-containing PCR products resulting from carryover contamination will be eliminated by UNG
1	95°C	15 min	Initial PCR activation step	HotStarTaq DNA Polymerase is activated by this step
2-step cycling:				Optimal performance is only assured using these cycling conditions
45	94°C	1 min	Denaturation	
	60°C	1 min	Annealing and extension	Combined annealing and extension step with fluorescence data collection

7. **Place the PCR tubes in the real-time cycler, and start the PCR cycling program.**
8. **Perform data analysis.**

   Before performing data analysis, select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification of data.

   The internal control will be always detectable, including in the no template control (NTC) sample.

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
Target signal not detected (or detected late), including target signal in positive controls and/or internal control in NTCs	
a) Incorrect cycling conditions	Always start with the optimized cycling conditions specified in the protocols.
b) HotStarTaq DNA Polymerase not activated	Ensure that the cycling program includes the HotStarTaq DNA Polymerase activation step (15 min at 95°C) as described in the protocols.
c) Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers, probes, and template nucleic acid. Repeat the assay.
d) Incorrect or no detection step	Ensure that fluorescence detection takes place during combined annealing/extension step when using TaqMan probes.
e) Primer or probe concentration not optimal	Ensure the correct handling and storage of the Certal Residual DNA Assay and Certal Internal Control Assay (see "Shipping and Storage", page 4 and "Reconstitution of components", page 18).
f) Insufficient number of cycles	Increase the number of cycles.
g) Incorrect detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for each reporter dye. Check whether the chosen combination of reporter dyes is compatible with the selected detection channels or filter sets.

	Comments and suggestions
h) Incorrect detection channel/filter chosen	Ensure that the correct detection channel is activated or the correct filter set is chosen for each reporter dye. Check whether the chosen combination of reporter dyes is compatible with the selected detection channels or filter sets.
i) No detection of Certal Internal Control and Certal Residual DNA Positive Control	Check the concentration and storage conditions of the control nucleic acids (see "Shipping and Storage", page 4 and "Reconstitution of components", page 18). If necessary, make new serial dilutions of control nucleic acid from the stock solutions. Repeat the assay using the new dilutions. Ensure that sufficient copies of the target nucleic acids are present in your positive control samples.

Target signal and IC signal not detected (or detected late) in samples, while target signal is detected in positive control and internal control signal is detected in NTCs

Presence of inhibitory substances or nucleases in the samples	Check the concentration, storage conditions, and quality of the starting template nucleic acids. Efficient removal of PCR inhibitors is essential for optimal results. Purify nucleic acids from your sample using the QIAsymphony Certal Extraction Kits; see ordering information for details. Samples containing low amounts of inhibitors may still be used successfully in qPCR by adding less sample (and therefore less inhibitor) to the reaction. Add a smaller sample volume to the reactions or dilute samples (e.g., 5- and 10-fold) before use in PCR. Ensure that all reagents, buffers, and solutions used for isolation and dilution of template nucleic acids are free of nucleases.

IC signal is detected late or not detectable in samples, while target signal is detected in the samples with early C_T values

Template amount too high	When signals are coming up at very early C_T values, make serial dilutions of the sample. Repeat the assay using the dilutions.

	Comments and suggestions
No linearity in ratio of C_T value/crossing point to log of the template amount	
a) Template amount too high	When signals are coming up at very early C_T values, adjust the analysis settings accordingly. Dilute sample so that it is in the linear range of the assay.
b) Template amount too low	Increase template amount if possible. Note that detection of very low starting copy numbers may not be in the linear range of a standard curve.
Increased fluorescence or C_T value for genomic DNA in "No Template" control	
a) Contamination of reagents	Discard all the components of the duplex assay (e.g., master mix, primers, and probes, controls). Repeat the Certal Residual DNA Detection Assay using new components.
b) Contamination during reaction setup	Take appropriate safety precautions (e.g., use filter tips). Use uracil-N-glycosylase to prevent carryover of PCR products from previous reactions.
c) Minimal probe degradation, leading to sliding increase in fluorescence	Check the amplification plots, and adjust the threshold settings.
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.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Appendix A: Data Analysis

When carrying out data analysis, follow the recommendations provided by the manufacturer of the real-time cycler. Fundamental guidelines for data analysis and some important considerations are given below. Further information can be found in the *Critical Factors for Successful Real-Time PCR* Brochure. To obtain a copy, contact QIAGEN Technical Services, or visit www.qiagen.com/literature/brochures to download a PDF.

Considerations for duplex data analysis

Real-time PCR data are produced as sigmoidal-shaped amplification plots (when using a linear scale), in which fluorescence is plotted against the number of cycles (Figure 2, page 35).

- The threshold cycle (C_T value) serves as a tool to calculate the starting template amount in each sample. This is the cycle in which the first detectable significant increase in fluorescence is observed.
- The method for determination of C_T values differs depending on the real-time cycler used. Check the handbook or the software help file for your real-time cycler for details on threshold settings.
- Most real-time cyclers contain a function that determines the noise level in early cycles, where there is no detectable increase in fluorescence due to PCR products (usually referred to as the baseline settings). Adjust the settings for this function.
- For duplex assays, the analysis settings need to be adjusted for each of the reporter dyes used.

Data interpretation of residual DNA and Certal Internal Control detection

When performing PCR using unknown samples, we recommend including appropriate controls in each run. This includes the addition of the Certal Internal Control to the reaction and the use of a positive control to verify negative test results. The controls are provided with the Certal Residual DNA Kit. For more information, see "Controls", page 17. Generally, the Certal Internal Control will be detected with similar C_T values even when used with a wide range of target amounts (Figure 2).

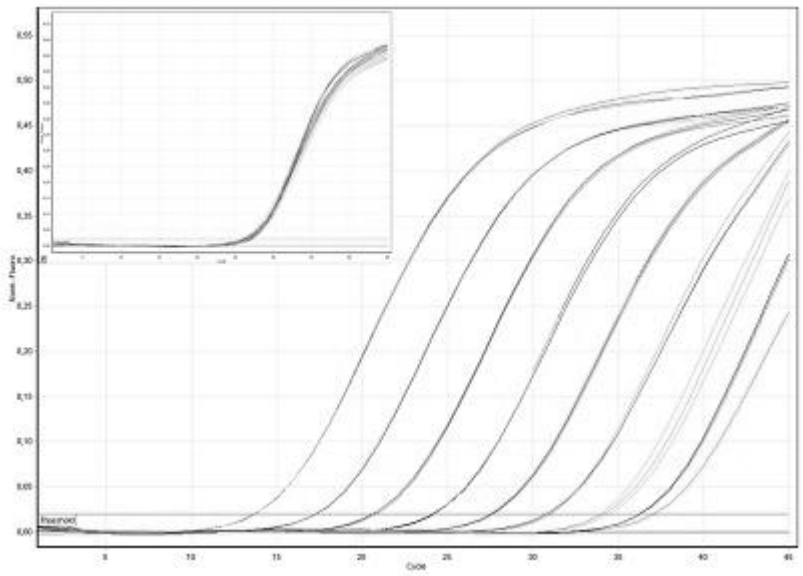


Figure 2. Sensitive and reliable detection of CHO genomic DNA on the Rotor-Gene Q. The reliability of the Certal Residual DNA Assay on the Rotor-Gene Q is demonstrated by evenly spaced amplification plots for the target signal and stable C_T values for the internal control (inset), which indicate that sensitive detection of the internal control is not affected by the wide range of CHO genomic DNA concentration.

Possible results for qualitative residual DNA detection:

A signal for the residual DNA target is detected in the sample.

The result of the analysis is positive: the sample contains targeted host cell DNA. In this case, the detection of a signal for MAX is dispensable. Note that high initial concentrations of target host cell DNA resulting in a strong positive signal for residual genomic DNA may lead to a reduced or absent fluorescence signal of the Certal Internal Control (MAX signal) due to competition.

In the sample, no signal is detected for the target residual DNA. At the same time, a signal for the Certal Internal Control appears

This indicates that no residual target DNA is detectable in the sample. It can be considered negative, provided that a positive control reaction has been performed and proven the functionality of the Certal Residual DNA Detection assay. In the case of a negative residual DNA PCR, the detected signal of the Internal Control rules out the possibility of PCR inhibition.

No signal is detected for target residual DNA and Certal Internal Control

If no signals for the target residual DNA and the Certal Internal Control are detected in the sample, positive control, and NTC, the PCR run may have failed. Information regarding error sources and their solution can be found in “Troubleshooting Guide”, page 30.

If a signal for the target residual DNA is detected in the positive control, and a signal for the Certal Internal Control is detected in the NTC, but no signals for the residual target and Certal Internal Control are detected in the sample, this indicates that inhibition may have occurred in the sample reactions. Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 30.

Appendix B: Isolation and quantification of residual genomic DNA

Genomic DNA preparation and quality

Since PCR consists of multiple rounds of enzymatic reactions, it is more sensitive to impurities such as proteins, phenol/chloroform, salts, and EDTA than single-step enzyme-catalyzed reactions. Purity of nucleic acid templates is particularly important for real-time PCR, since contaminants can interfere with fluorescence detection. QIAGEN offers a complete solution for residual genomic DNA purification that provides the highest-quality templates for real-time PCR:

- QIAsymphony Certal Residual DNA Kit and QIAsymphony Certal Vaccine NA Kit — for purification of residual host cell DNA and viral NA from bioprocess purification buffer, cell culture supernatant samples, and vaccine preparations using the QIAsymphony SP
- QIAsymphony SP/AS — for automated purification of gDNA from up to 96 samples, including assay setup

Determining concentration and purity of nucleic acids

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. For accuracy, absorbance readings at 260 nm should fall between 0.15 and 1.0. Brief guides to spectrophotometric and molar conversion values for different nucleic acid templates are listed in Tables 14 and 15.

Table 14. Spectrophotometric conversions for nucleic acid templates

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

* Absorbance at 260 nm = 1; 1 cm detection path.

Table 15. Molar conversions for nucleic acid templates

Genomic DNA	Size	pmol/µg	Molecules/µg
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.

Note that absorbance measurements cannot discriminate between DNA and RNA. Depending on the method used for template preparation, DNA may be contaminated with RNA, or RNA may be contaminated with DNA, and either of these will result in misleadingly high A_{260} values. It is particularly important to bear this in mind when preparing standards for absolute quantification.

The ratio between the absorbance values at 260 nm and 280 nm gives an estimate of the purity of DNA. To determine nucleic acid purity, we recommend measuring absorbance in 10 mM Tris·Cl,‡ pH 7.5. Pure DNA has an A_{260}/A_{280} ratio of 1.8–2.0. Lower ratios indicate the presence of contaminants such as proteins.

Nucleic acid quantification

Further information can be found in the *Critical Factors for Successful Real-Time PCR* brochure. To obtain a copy, contact QIAGEN Technical Services, or visit www.qiagen.com/literature/defaultbrochures.aspx to download a PDF.

Absolute quantification

The absolute amount of a target nucleic acid is determined using external standards. The sequence of the standards is usually the same as, or very similar to, the target sequence, but the primer binding sites of the standards must be identical to those in the target sequence. This ensures that both the standards and the target are amplified with equivalent efficiencies, which is essential for absolute quantification. A standard curve (plot of C_T value/crossing point against log of amount of standard) is generated using different dilutions of the standard. The target and each of the standards are amplified in separate tubes.

‡ 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), available from the product supplier.

The C_T value of the target is compared with the standard curve, allowing calculation of the initial amount of the target. It is important to select an appropriate standard for the type of nucleic acid to be quantified.

Generation of standard curves using genomic DNA

Standard curves are used for absolute quantification. To generate a standard curve, at least 5 different amounts (e.g., 300 pg to 30 fg) of the standard should be quantified, and the amount of unknown target should fall within the range of the standard curve. Reactions should be carried out in at least triplicate, especially when quantifying standards of low copy number.

For absolute quantification of DNA molecules, the copy number or concentration of the nucleic acids used as standards must be known. Use the same cell-line as in your process samples to ensure correct quantification. This is important because cell-lines may differ in the grade of (partial) polyploidy and repetitive sequences due to genomic rearrangements.

Calculation of genomic copy number

The copy number of the target present in the genomic DNA can be directly calculated if the genome size of the organism is known.

Example organism: *Mus musculus*

Genome size (haploid): 2.7×10^9 bp

Molecular weight: 1.78×10^{12} Daltons

1.78×10^{12} g genomic DNA corresponds to 6.022×10^{23} copies of a single-copy gene. 1 µg genomic DNA corresponds to 3.4×10^5 copies of a single-copy gene.

Genomic standard DNA dilution series

- Dilute the genomic standard DNA to a final concentration of 225 ng/µl (corresponds to 30 ng in 7.5 µl).
- Dilute the genomic DNA to begin the dilution series with 30 ng of genomic DNA.
- Perform a 10-fold dilution series in QuantiTect Nucleic Acid Dilution buffer as described in Table 16, ranging from 30 ng to 30 fg.
- Use at least 5 amounts in the expected range of the sample (e.g., 300 pg to 30 fg) to allow a proper estimation of the quantity of residual target genomic DNA.

Table 16. Dilution series for genomic standard DNA

Concentration in 7.5 µl	Genomic DNA (µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
30 ng	10 µl	90 µl
3 ng	10 µl	90 µl
300 pg	10 µl	90 µl
30 pg	10 µl	90 µl
3 pg	10 µl	90 µl
300 fg	10 µl	90 µl
30 fg	10 µl	90 µl
3 fg	10 µl	90 µl

Calculation of the amount of host cell DNA

Prepare a standard curve with a 10-fold dilution series of purified genomic DNA from your cell line in the expected range of your target sample according to Table 16. Comparison with the dilution series using the genomic DNA of the identical cell line as an absolute standard allows the calculation of the residual DNA.

Appendix C: Rotor-Gene Q Setup for Adjustment of Fluorescence Channel Sensitivity

Adjustment of fluorescence channel sensitivity for the Certal Internal Control Assay

We recommend setting the detection range of the yellow channel for the Certal Internal Control Assay on a fixed gain to ensure optimal fluorescence gain. Click "Edit Gain" in the "New Run Wizard" dialog box (Figure 3) to open the "Gain for Yellow" dialog box. Set the gain for the yellow channel to a value of 9 (Figure 3).

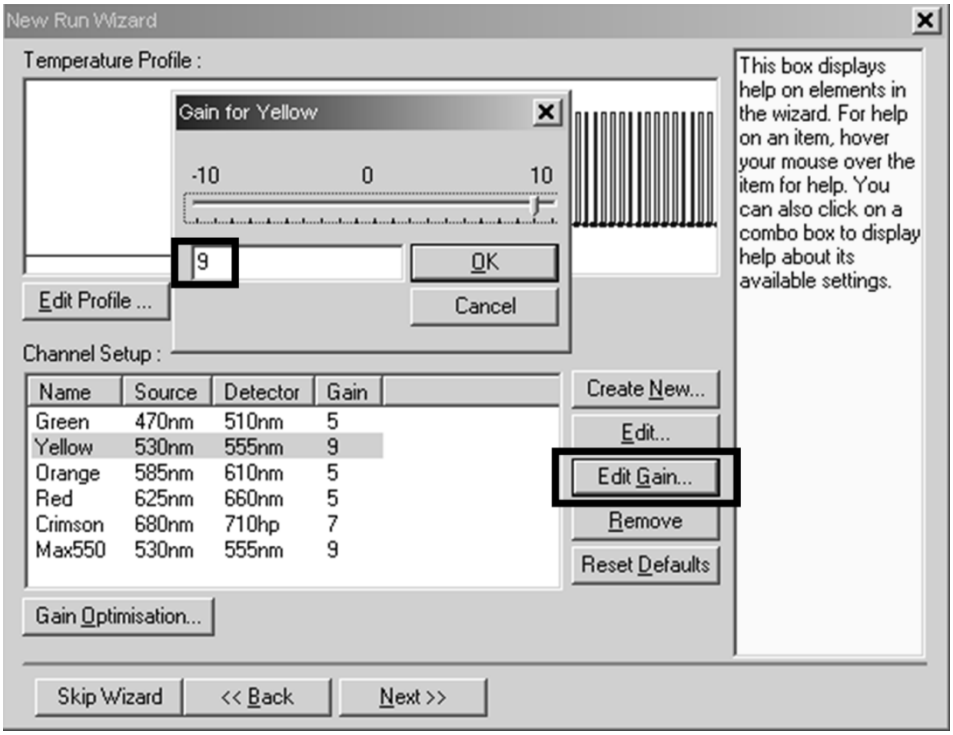
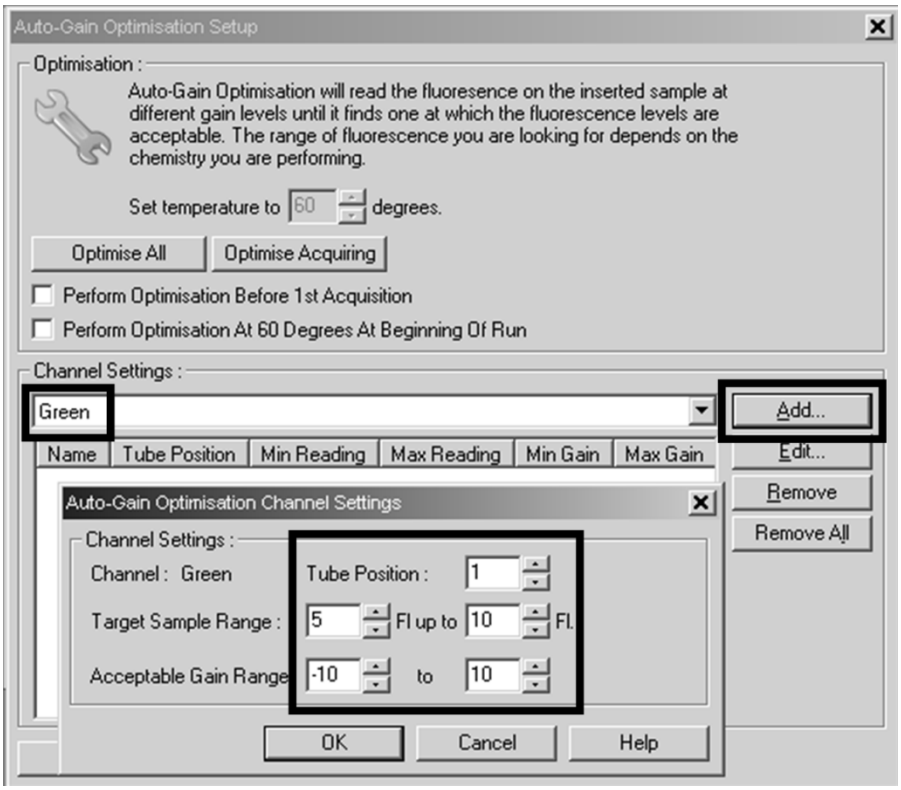


Figure 3. Setting a fixed gain for the Certal Internal Control Assay (yellow channel).

Adjustment of fluorescence channel sensitivity for the residual genomic DNA assay

We recommend determining the detection range of the green channel for the pathogen assay according to the fluorescence intensities in the PCR tubes. Click "Gain Optimisation" in the "New Run Wizard" dialog box (Figure 4) to open the "Auto-Gain Optimisation Setup" dialog box. Add channel "Green" from the drop-down menu and adapt the "Auto-Gain Optimisation Settings" as shown in Figure 4A). Adjust the calibration temperature to 60 degrees to match the annealing temperature of the amplification program, and check the box "Perform Optimisation Before 1st Acquisition" (Figure 4B).

A



B

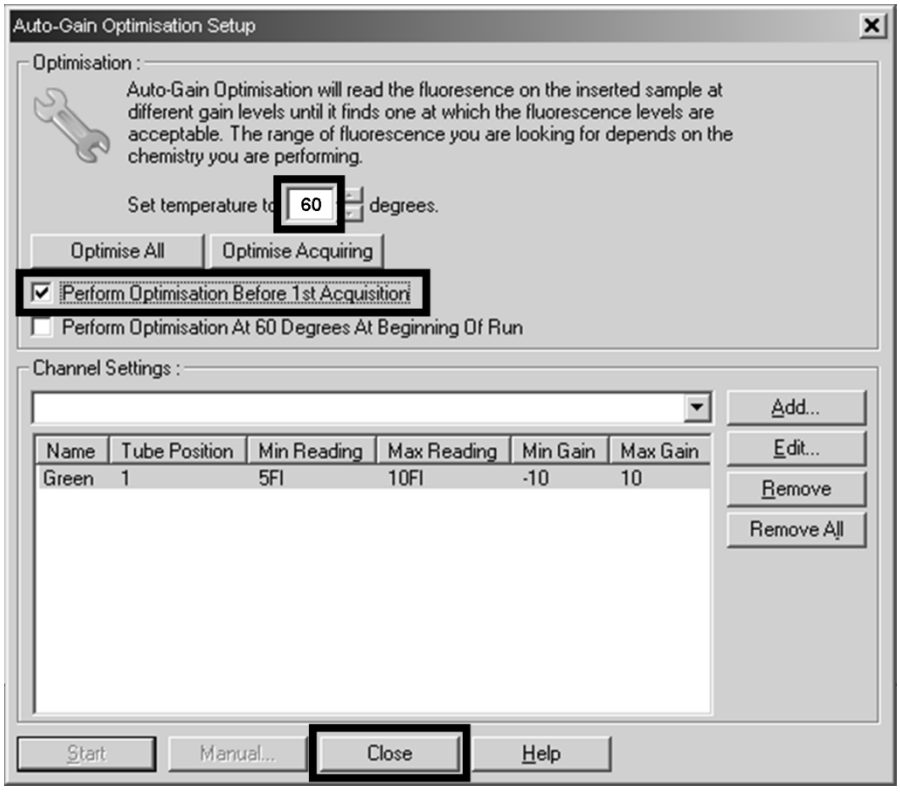


Figure 4. Adjusting the fluorescence channel sensitivity for the pathogen assay (green channel) in the "Auto-Gain Optimisation Setup" dialog box.

Ordering Information

Product	Contents	Cat. no.
Certal CHO Detection Kit (100)	Reagents and controls for 100 x 25 µl reactions: 1.25 ml Certal ResDNA PCR Master Mix, 100 µl Uracil-N-Glycosylase (1U/µl), Certal Internal Control DNA and Assay, CHO DNA Positive Control and CHO-Assay, 210 µl High ROX dye solution, 210 µl ROX dye solution, 3 ml Nucleic Acid Dilution Buffer, 1.9 ml TE Buffer	211822
Related products		
QIAsymphony Certal Residual DNA Kit (96)	For up to 96 preps of 500 µl each; includes 2 reagent cartridges and enzyme racks and accessories.	931855
QIAsymphony Certal Vaccine NA Kit (96)	For up to 96 preps of 500 µl each; includes 2 reagent cartridges and enzyme racks and accessories.	931955
Real-time cycler		
Rotor-Gene Q System	Real-time PCR cycler and High-Resolution Melt analyzer with up to 6 channels.	Varies
Automated purification and assay setup		
QIAsymphony SP/AS	Instrument for fully integrated sample preparation and assay setup.	Varies

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.

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