Quick-Start Protocol March 2016

Certal® Residual DNA Detection Kits

Certal Residual DNA Detection Kits (cat. nos. 211822 and 211842) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light.

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 if not otherwise stated on label.

The Certal Residual DNA Assay (FAM™ labeled), the Certal Internal Control Assay (MAX™ labeled) and the Certal Internal Control DNA should be stored at –30 to –15°C, either lyophilized or reconstituted. The Certal Residual DNA Positive Control should be stored at –30 to –15°C. Reconstitute the Certal Internal Control DNA soon after receipt. Avoid repeated (>6 times) freeze–thaw cycles of Certal Positive Control DNA and Certal Internal Control DNA.

Further information

- *Certal Residual DNA Detection Handbook*: **www.qiagen.com/HB-0978**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes 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.
- Be sure to activate the detector for each reporter dye (FAM and MAX) used. Depending on the instrument, it may also be necessary to perform a calibration procedure for each of the reporter dyes before they are used for the first time.

- 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 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®).
- If using for the first time, reconstitute the Certal Residual DNA Assay, Certal Internal Control Assay and Certal Internal Control DNA according to Table 1.
- 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 1. Product reconstitution

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

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, 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.
2. Prepare Certal Residual DNA Positive Control mix by adding 0.5 µl Certal Residual DNA Positive Control to 7 µl Nucleic Acid Dilution Buffer.
3. Prepare a reaction mix according to Table 2.
4. Mix the reaction mix thoroughly (by pipetting up and down or pulse vortexing), then dispense appropriate volumes into PCR tubes, plates or Rotor-Disc®.
5. Add 7.5 µl of the template gDNA or positive control DNA mix (from step 2) to the individual PCR tubes, wells of a PCR plate or wells of a Rotor-Disc.

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

Table 2. Reaction mix setup

Component	Cyclers not requiring ROX dye		Cyclers requiring ROX dye		
	Rotor-Gene® cyclers and 96-well cyclers	384-well cyclers	ABI 7500 /ViiA™ 7	ABI 7900, other ABI instrument 96-well	ABI 7900, other ABI instrument 384-well
2x Certal ResDNA PCR Master Mix	12.5 µl	10.0 µl	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
Certal Residual DNA Assay	0.5 µl	0.4 µl	0.5 µl	0.5 µl	0.4 µl
Certal Internal Control Assay	0.5 µl	0.4 µl	0.5 µl	0.5 µl	0.4 µl
Certal Internal Control DNA	0.5 µl	0.5 µl	0.5 µl	0.5 µl	0.5 µl
RNase-free water	3.25 µl	1.0 µl	2.75 µl	2.75 µl	0.6 µl
Template DNA (positive control or sample)	7.5 µl	7.5 µl	7.5 µl	7.5 µl	7.5 µl
UNG (1U/µl) (recommended)	0.25 µl	0.2 µl	0.25 µl	0.25 µl	0.2 µl
Total volume/reaction	25 µl	20 µl	25 µl	25 µl	20 µl

6. Program the real-time cycler according to Table 3.

   Note: Data acquisition should be performed during the combined annealing/extension step. Consult the real-time cycler 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] and internal control [MAX], which can serve as a VIC®, JOE® or HEX™ replacement without the need for an extra calibration step). For the Rotor-Gene Q real-time cycler, use the green channel for FAM and the yellow channel for MAX.

Table 3. Cycling conditions

Cycles	Temperature	Time (Rotor-Gene Q)	Time (block cyclers)	Step	Additional comments
1	50	2 min	2 min	UNG treatment (recommended)	Eliminates any dUMP-containing PCR products resulting from contamination due to carryover of PCR products
1	95	15 min	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	30 s	1 min	Denaturation	
	60	1 min	1 min	Annealing and extension	Combined annealing and extension step with fluorescence data collection

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

   Note: 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.

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