EUROPE
LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM • Tel.: +32 4 372 74 00
• Fax: +32 4 372 75 00 • Toll-free: + 800 666 00 123 • info@eurogentec.com
www.eurogentec.com

NORTH AMERICA
ANASPEC - 34801 Campus Drive • Fremont, CA 94555 • USA • Tel.: +1-510-791-9560
• Toll-free: +1 877 387 6436 • Fax: +1 510-791-9572 • service@anaspec.com
www.anaspec.com

Universal Exogenous qPCR Positive Control for qPCR Assays (Cy®5-QXL®670 Probe) Kit

RT-IPCC-Q02 (200 rxns) • RT-IPCC-Q10 (1000 rxns)

Introduction

Real-Time PCR assays are prone to inhibition by various substances found in many samples (clinical, soil, plant and other samples). Carryover of reagents used for the isolation of nucleic acids can also inhibit amplification reactions. Other causes of false-negative results include target nucleic acid degradation, sample processing errors and thermal cycler malfunction.

Eurogentec's Universal Exogenous qPCR Positive Control for probe-based Assays is an **optimised qPCR control** that was designed to distinguish true target negatives from false negatives due to PCR inhibition, incorrect pipetting or cycling parameters.

- The optimised control can be spiked into samples without compromising amplification efficiency of the target sequence.
  - A negative call for the target sequence combined to a positive call for the IPC indicates that no target sequence is present
  - A negative call for the target sequence and for the IPC suggests PCR inhibition or a reaction setup/cycling error.
- The optimised control doesn't match with any sequence routinely found in a lab
- The optimised control is detected using a Cy®5 -labelled probe and the target template is detected using a FAM-labelled probe.
- Avoid amplification of endogenous genes

Alternatively, the Universal Exogenous qPCR Positive Control may be used in standardised conditions as **extraction yield calibrator (a)**, **template quality sensor (b)** or **inter-run calibrator (c)**.

(a) A given quantity of control can be spiked into samples before extraction. A relative (directly comparing samples) or an absolute (using a dilution curve of the control) quantification is performed after extraction to normalize the extraction yields of the samples.

(b) Quantitative results of the spiked control within the template or within a reference buffer (pure water, reference template…) are compared in order to reject templates where PCR inhibition is high (low quality).

(c) Add a dilution series of the optimised control on each plate and use it to normalize PCR efficiencies between plates (also for cycler to cycler data normalization).

Kit contents and Storage

Component	References	Volume	Description
10X EGT IPC mix	RT-IPCC-Q02	1100 µl (Black cap)	Tube containing IPC primers and Cy®5-QXL®670 probe
	RT-IPCC-Q10	5 x 1100 µl (Black cap)	
50X EGT IPC DNA	RT-IPCC-Q02	220 µl (red cap)	Tube containing IPC template DNA
	RT-IPCC-Q10	1100 µl (yellow cap)	

- For long-term storage, the Universal Exogenous qPCR Positive Control should be kept in the dark, at -20 °C in a constant temperature freezer.
- For short-term storage, the Universal Exogenous qPCR Positive Control can be kept in the dark, at 4 °C to 6°C for one month.
- The 10X IPC Mix should be protected from light whenever possible to avoid degradation of the probe.
- Avoid multiple freeze-thaw cycles.

Protocol

1. Thaw all required reagents completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipetting.
2. Prepare the reaction mix.
   Add all components together, except for the template.

Reagent	Volume		Volume for 100 reactions(1)	
2X qPCR MasterMix (optimised with Eurogentec mixes)	25 µl		2500 µl	
	Target Ct <30	Target Ct >30 (2)	Target Ct <30	Target Ct >30 (2)
10X IPC mix (black cap)	5 µl	1 µl	500 µl	100 µl
50X IPC DNA (3)	1 µl	0.5 µl	100 µl	50 µl
Target primers, probe & deionized water (4)	14 µl	18.5 µl	1400 µl	1850 µl
(Template DNA)	*(5 µl)*		*(500 µl)*	
Total volume	**50 µl**		**5000 µl**	

(1) To correct for dispensing losses prepare an excess of reaction mix (for example, a 100 reactions mix for 96 reactions)
(2) If target Ct >30 (low copy), use this optimised IPC mix and DNA concentrations to reach optimal duplex results.
(3) For negative IPC control (IPC-), replace the IPC DNA by water.
(4) Add water instead of target specific primers and probe if the IPC positive control is run in separated wells.

3. Mix thoroughly by inversion. Spin down.
4. Pipette 5µl of the template DNA for your samples and 5µl of water or buffer for your negative control into your PCR tubes / PCR plate. Adjust the water volume in the table here above if the template volume is different than 5 µl.

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5. Add 45µl of the reaction mix per tube / well, close the tube / plate, mix gently on a stirrer and spin down. Ensure that no bubbles are present in the reaction tubes / wells.
6. Program the Real-Time thermocycler using qPCR kit manufacturer recommended parameters.

For example:	
UNG Step (if necessary)	2 min at 50°C
HotGoldStar activation	10 min at 95°C
40 cycles	15 sec at 95°C
	1 min at 60°C (fluorescence reading / except if end-point Plate Read Detection is performed for result calling)

Note :
- For Cy®5 dye, please use the ROX® or the Cy®5 filter depending on your thermocycler.
- Stratagene Mx3000p filter set gain: 4x for HEX, 1x for ROX (if ROX passive reference is present), 4x for FAM, 1x for Cy®5.

Interpreting IPC results

The Universal Exogenous qPCR Positive Control, in conjunction with your target system, allows you to identify samples that are positive and negative for a specific target sequence.

During amplification, the sample and IPC generate reporter fluorescence signals such that identification calls may be made on unknown samples. Positive and negative calls are made on the basis of statistical analysis of data from the two dye layers. The statistical analysis should be based on threshold values for positive FAM and Cy®5 calls on the basis of the No Template Control (NTC ; FAM neg.) and the Negative Positive Control (IPC– ; see note 3 from table 1) baselines.
Automatic calls can be made using Plate Read functions – based on end point detection - available on some thermocyclers. Follow the manufacturer recommendations for automatic calling of unknown samples.

Target Amplification (FAM channel)	IPC amplification (Cy®5 channel)	Target result is
Positive	Positive (*)	Positive
Negative	Positive	Negative (no target sequence)
Negative	Negative	No conclusion on target presence (**)

* In the presence of a very strong signal for the target assay, low or no signal can be observed for IPC amplification (Cy®5 layer). This is due to the limiting IPC DNA and primers concentrations in the assay.

** If the IPC amplification is negative, as the target amplification, this suggests the presence of inhibitors, a wrong PCR set-up, a defective mix or thermal cycling protocol. Carefully check individual components and steps, then try again with diluted sample to subdue the impact of inhibitors.

When used as extraction yield calibrator or template quality sensor, the spiked validated control co-purifies and co-amplifies with the target nucleic acid and serves as sensitive indicator of loss or degradation of the target during sample processing or inhibition of amplification due to poor quality sample. It provides an accurate way to assess the integrity of all the steps in a nucleic acid amplification assay.

When used as inter-plate data calibrator, a dilution series of the positive control is added on every plate, at a fixed position. The corresponding data serves as reference for plate-to-plate normalization. Note that some commercial qPCR data-analysis softwares offer inter-plate calibration capabilities.

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