qPCR DNA Extraction and Inhibition Control
[Yakima Yellow®-BHQ-1® Probe] 200 RXNs kit

Introduction

Eurogentec's Sample Processing Control (SPC) is an optimized Taqman® control designed to be used as qPCR DNA Extraction and Inhibition Control.

- The optimized control doesn't match with any sequence routinely found in a lab
- The optimized control is detected using a Yakima-Yellow® (VIC® equivalent)-labelled probe
- Avoid amplification of endogenous genes.

As Sample Processing Control, a given quantity of control DNA is spiked into samples before extraction without compromising amplification efficiency of the target sequence. A direct comparison of SPC results between samples allows distinguishing true target negatives from false negatives due to poor extraction yield, PCR inhibition, incorrect pipetting or cycling parameters.

When comparing extraction methods, a given quantity of control DNA is spiked into the reference sample before extraction. Extraction yields of the different tested methods are compared by relative comparison (directly comparing methods) or by absolute quantitation (using a dilution curve of the control) after extraction.

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

Kit content and storage

Component	Kit Reference	Volume	Description
10 X EGT Control Mix	RT-SPCY-B02	1100 µL [white cap]	Tube containing primers for the control and YY-BHQ-1® probe
	RT-SPCY-B10	5 x 1100 µL [white cap]	
EGT Control DNA	RT-SPCY-B02	220 µL [Green Cap]	Sample Processing Control
	RT-SPCY-B10	1100 µL [Blue Cap]	

Simplified Protocol for the Sample processing Control assay

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. Add 1 µL of the validated SPC[1] dilution into your sample before extraction.
   - Before first use of the SPC, prepare a 1/10th and a 1/100th dilution of the control DNA in pure water and store them on ice*.
   - Evaluate the non-diluted and the 2 diluted control DNA solutions in separate extractions by adding 1 µL of control DNA[1] into your reference sample before parallel extractions. Perform steps 2 to 5 and select the dilution factor generating Cq values comprised between 30 and 33. Evaluate a higher dilution in case Cq comes out below 30 for the 1/100th dilution.

3. Perform extraction.

* Always use low-binding tubes when working with diluted DNA samples, e.g. control DNA.

Notes:
[1] For negative control (SPC), replace DNA by water. For Low copy detection, a 10 times dilution can be used when first evaluating the control.

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4. Prepare the qPCR reaction mix [2].

Reagent	Volume per well[2]
2 X qPCR Master Mix [optimized with Eurogentec Mixes]	12.5 µL
10X Control Mix [white cap]	2.5 µL
Customer target primers and Probe Deionized water Extracted DNA template [target DNA + Control DNA]	10 µL
Total Volume	25 µL

5. Program the Real-Time thermocycler using qPCR kit manufacturer recommended parameters.[3-4]

Example:
UNG Step (if necessary) ..... 2 min at 50°C
Taq activation ..... 3 to10 min at 95°C (according to your MasterMix)
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)

Interpreting SPC results

The spiked SPC co-purifies during extraction and co-amplifies with the target nucleic acid. In conjunction with your target system, the SPC allows you to identify positive and negative samples for a specific target sequence. During amplification, the sample and SPC generate reporter fluorescence signals. 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 VIC® calls on the basis of the No Template Control (NTC; FAM neg.) and the Negative Control (SPC-; Yakima Yellow neg.) 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.

– A negative call for the target sequence combined to a positive call for the SPC indicates that no target sequence is present

– A negative call for the target sequence and for the SPC suggests PCR inhibition, a reaction setup/ cycling error or extraction failure.

Target Amplification (FAM channel)	SPC Amplification	Target result is
Positive	Positive[5]	Positive
Negative	Positive	Negative (no target sequence)
Negative	Negative	No conclusion on target presence [6]

In case of negative SPC results, repeat the qPCR steps 4-5 with [a] and without [b] direct addition of **an appropriate volume** of diluted SPC [see Protocol step 2 for dilution set-up].

$$\text{SPC Appropriate Volume} = \frac{\text{Sample Volume used for the qPCR Assay}}{\text{Sample Total Volume after Extraction}} \times \text{SPC Volume spiked before extraction}$$

– A positive result in assays [a] and [b] suggests assay setup errors or cycling parameter / cycler default during initial assay.
– A positive result in assay [a] suggests a failed extraction.
– A negative result in assays [a] and [b] suggests the presence of PCR inhibitors in the extracted sample.
The SPC provides an accurate way to assess the integrity of all the steps in a nucleic acid amplification assay.

Notes:
[2] To correct for dispensing losses prepare an excess of reaction mix (for example, a 100 reactions mix for 96 reactions).
[3] For Yakima Yellow® dye, please use the VIC® or the HEX filter depending on your thermocycler.
[4] Stratagene Mx3000p filter set gain: 4x for HEX, 1x for ROX (if ROX passive reference is present), 4x for FAM.
[5] In the presence of a very strong signal for the target assay, low or no signal can be observed for SPC amplification (VIC® layer). This is due to the limiting control DNA and primers concentrations in the assay.
[6] If the SPC amplification is negative, as the target amplification, this suggests the presence of inhibitors, an extraction failure, a wrong PCR set-up, a defective mix or thermal cycling protocol. Carefully check individual components and steps, then test with diluted sample to subdue the impact of inhibitors.

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