Universal Exogenous qPCR Positive Control for TaqMan Assays
(FAM-BHQ1™ Probe) 100 rxns Kit
RT-IPCF-B01

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 TaqMan® Assays is an optimised Taqman® 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 FAM® probe and the target template is detected using a labelled probe in another channel.
- 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 cyclers to cyclers data normalization).

Kit contents and Storage

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X EGT IPC mix</td>
<td>550 µl</td>
<td>1 Tube (green cap) containing IPC primers and FAM-BHQ1™ probe</td>
</tr>
<tr>
<td>50X EGT IPC DNA</td>
<td>110 µl</td>
<td>Tube (red cap) containing IPC template DNA</td>
</tr>
</tbody>
</table>

- 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Volume for 100 reactions(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X qPCR MasterMix (optimised with Eurogentec mixes)</td>
<td>Target Ct &lt;30</td>
<td>Target Ct &gt;30(2)</td>
</tr>
<tr>
<td>10X IPC mix (green cap) (3)</td>
<td>5 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td>50X IPC DNA (red cap) (4)</td>
<td>1 µl</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>Target primers, probe &amp; deionized water (5)</td>
<td>14 µl</td>
<td>18.5 µl</td>
</tr>
<tr>
<td>(Template DNA) (5 µl) (500 µl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total volume</td>
<td>50 µl</td>
<td>5000 µl</td>
</tr>
</tbody>
</table>

(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.

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.
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
- Takyon activation 3 min at 95°C
- 40 cycles 15 sec at 95°C
  30 sec at 60°C (fluorescence reading / except if end-point Plate Read Detection is performed for result calling)

### 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 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.

<table>
<thead>
<tr>
<th>Target Amplification (FAM channel)</th>
<th>IPC amplification (VIC/YY channel)</th>
<th>Target result is</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Negative (no target sequence)</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>No conclusion on target presence (**)</td>
</tr>
</tbody>
</table>

* In the presence of a very strong signal for the target assay, low or no signal can be observed for IPC amplification (FAM 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.