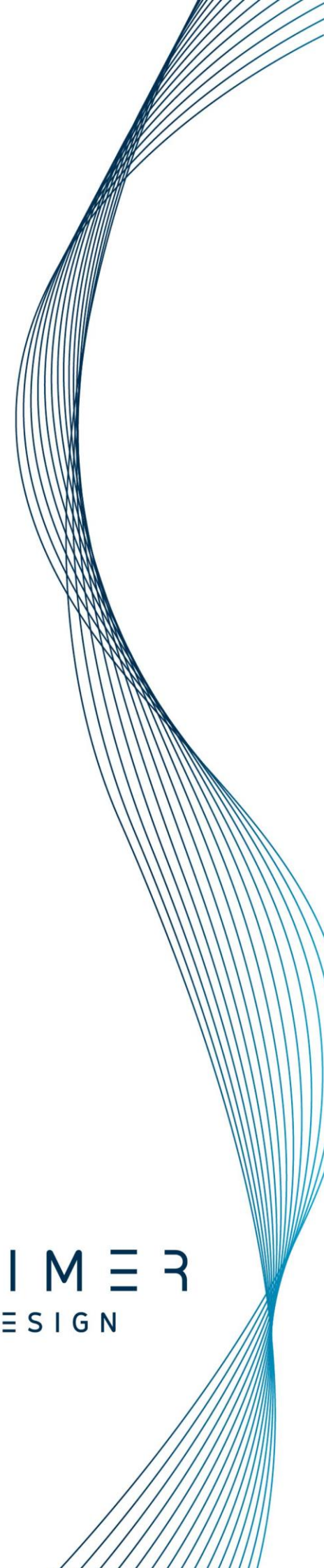
Precision® qPCR endogenous control

Instructions for use of Primerdesign Precision qPCR endogenous control for real-time PCR



PRIMER DESIGN

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Introduction

When performing a PCR test, it is advantageous to confirm extraction of a valid biological template to allow accurate data analysis. The Primerdesign Precision qPCR endogenous control kit, contains a primer and probe mix designed to detect an endogenous gene in your species of interest. Detection of the endogenous control in your test sample confirms a high quality starting biological sample. Whereas a poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Kit Contents

- **Precision® qPCR endogenous control primer/probe mix (**150 reactions**, BROWN)**
- **RNase/DNase free water (WHITE)**

Recommended Accompanying Products

- **genesig® pathogen detection kits**
- **Primerdesign real-time PCR internal control**
- **Primerdesign BrightWhite real-time PCR plasticware**

Reagents and Equipment to Be Supplied by User

- **Real-time PCR Instrument**
- **Pipettors and tips**
- **Vortex and centrifuge**

Kit Storage

The Primerdesign Precision qPCR endogenous control kit should be stored at -20ºC on arrival. Repeated freeze/thawing should be avoided as it may compromise the performance of the product. Under these conditions reagents are stable for twelve months from date of purchase.

Suitable Sample Material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase free water.

Licensing Agreement and Limitations of Use

PCR is covered by several patents owned by Hoffman-Roche Inc and Hoffman-LaRoche Ltd. Purchase of Primerdesign kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

Primerdesign Ltd Satisfaction Guarantee

Primerdesign takes pride in the quality of all our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, Primerdesign will replace the item free of charge.

Quality Control

As part of our ISO9001 and ISO13485 quality assurance systems, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.

Bench-side Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend lyophilised components in RNase/DNase free water provided**.
   To ensure complete resuspension, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use.

Components	Volume
Precision qPCR endogenous control primer/probe mix (**BROWN**)	165 µl

Real-time PCR detection protocol

1. For each DNA sample prepare an endogenous control reaction mix.

When using Primerdesign oasig, PrecisionPLUS or PrecisionFAST Master Mix, make up a mix containing all reagents according to the protocol below:

Components	1 Reaction
Oasig/PrecisionPLUS/PrecisionFAST Master Mix	10 µl
Precision qPCR endogenous control primer/probe mix	1 µl
Template DNA (25ng)	x µl
RNase/DNase free water (up to Final volume)	x µl
Final volume	**20 µl**

Or if using Master Mix from another supplier, make up a mix containing all reagents according to the protocol below:

Components	1 Reaction
User supplied Master Mix	x µl
Precision qPCR endogenous control primer/probe mix	1 µl
Template DNA (25ng)	x µl
RNase/DNase free water	x µl
Final volume	**20 µl**

2. Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.

3. Prepare sample DNA templates for each of your samples by diluting in RNase/DNase free water (suggested concentration 5ng/µl).
If the concentration of DNA is not known, then dilute your DNA sample reactions 1:20 (10µl of sample DNA and 190µl of water).

4. Pipette 5µl of diluted template into each well, according to your experimental plate set up.
For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. Proceed to amplification protocol.

Amplification Protocols

Amplification conditions using oasig or PrecisionPLUS 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme Activation	2 min	95ºC
Cycling x40	Denaturation	10 sec	95ºC
	DATA COLLECTION*	60 sec	60ºC

*Fluorogenic data should be collected during this step through the appropriate channel.

Amplification conditions using PrecisionFAST 2X qPCR Master Mix

	Step	Time	Temp
	Reverse Transcription	10 min	55ºC
	Enzyme activation	2 min	95ºC
Cycling x40	Denaturation	10 sec	95ºC
	DATA COLLECTION*	60 sec	60ºC

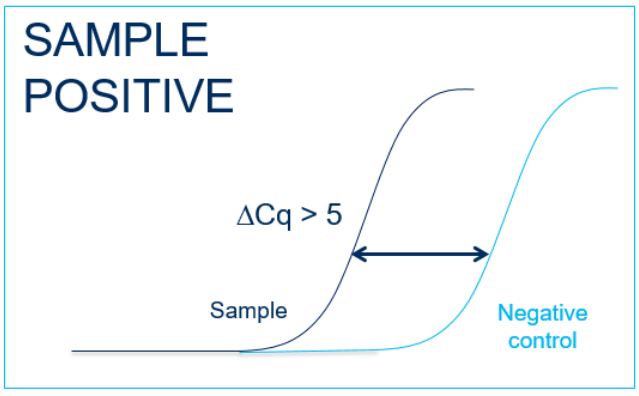
*Fluorogenic data should be collected during this step through the appropriate channel.

Interpretation of results

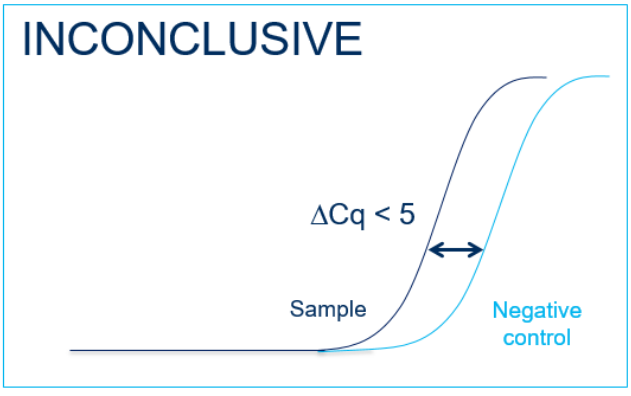
Precision qPCR endogenous controls should be used in conjunction with a detection assay (e.g. genesig kit, homebrew assay or a kit from an alternative supplier). In such circumstances the table below should serve as a guide to enable full analysis of sample data:

Target of Interest Assay (Sample)	**Precision qPCR Endogenous control** (Sample)	**Positive Control** (Target Assay)	**Negative Control** (Target Assay)	**Interpretation**
≤ 30	**+ / -**	**+**	**-**	**POSITIVE QUANTITATIVE RESULT** calculate copy number
> 30	**+**	**+**	**-**	**POSITIVE QUANTITATIVE RESULT** calculate copy number
> 30	**-**	**+**	**-**	**POSITIVE QUALITATIVE RESULT** do not report copy number as this may be due to poor sample extraction
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤35**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+ / -**	**+**	**>35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

* Where the test sample is positive and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.