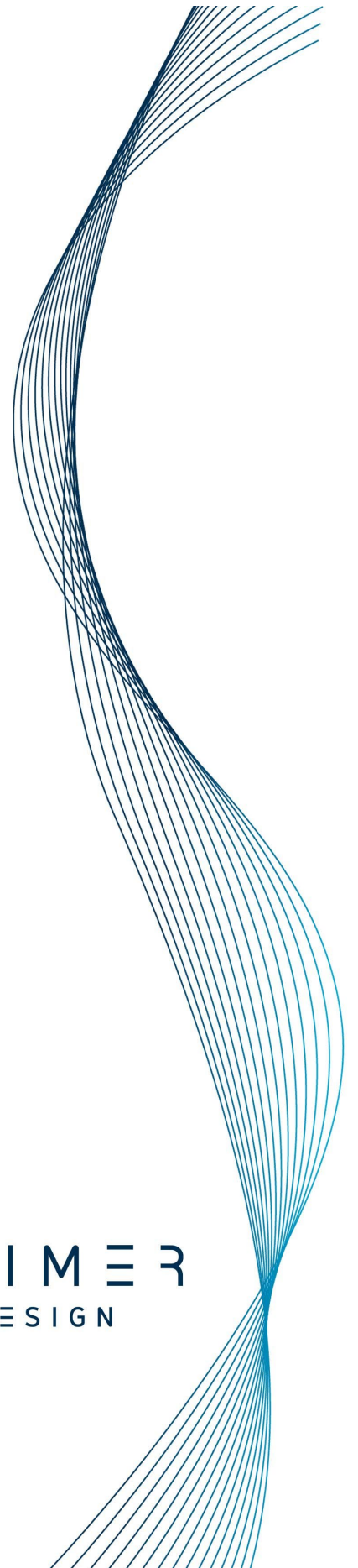


Internal DNA extraction control

Instructions for use of DNA real-time PCR
internal extraction control kit

PRIMER
DESIGN



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Introduction

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process (internal extraction control). Successful co-purification and real-time PCR amplification of the control DNA also indicates that PCR inhibitors are not present at a high concentration.

Alternatively, the DNA in this kit can be spiked directly into a PCR reaction to confirm directly that the test sample is competent to support qPCR amplification (internal PCR control).

A separate primer/probe mix is supplied with this kit to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target gene even when the target gene is present at low copy number. A number of different dyes are available so that a range of channels can be used to detect the control DNA. A dye should be selected that reads through a separate channel to the target gene.

Channel	Excitation Wavelength	Emission Wavelength
FAM	495	520
Cy5	649	670
TAMRA	555	576
VIC	530	549

The exogenous DNA sequence supplied with this kit can be used with all human and animal tissues except rat and when used according to the protocols, gives a Cq value of 28 ± 3 .

Kit contents

- **Lyophilised Internal control DNA template** (150 reactions, **BLUE**)
- **Lyophilised Internal control primer/probe mix** (150 reactions, **BROWN**)
- **RNase/DNase free water** (**WHITE**)
- **Template preparation buffer** (**YELLOW**)
for resuspension of internal control DNA template

Reagents and equipment to be supplied by user

- **Real-Time PCR instrument**
- **Master Mix or Master Mix components**
This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS 2X qPCR Master Mix or oasig 2X qPCR Master Mix.
If intending to use this kit with a FAST Master Mix, please contact Primerdesign for further information, support@primerdesign.co.uk
- **Pipettors and Tips**
- **Vortex and centrifuge**

Kit storage

This kit is stable at room temperature but should be stored at -20°C on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilised components have been resuspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

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 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

At Primerdesign our commitment to Quality is a fundamental part of our business and we proactively make improvements in our service and product quality whilst meeting all relevant standards.

Notices and disclaimers

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The purchase of the Primerdesign™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc or others.

Trademarks

Primerdesign™ is a trademark of Primerdesign Ltd.

Precision® is a registered trademark of Primerdesign Ltd.

oasig™ is a trademark of Primerdesign Ltd.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. ABI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applied Biosystems (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc.,

Bench-side protocol

To minimise 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 cabinet. Filter tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening.

This will ensure lyophilised primer/probe mix is in the base of the tube and is not spilt upon opening the tube.

2. Resuspend lyophilised primer/probe mix in the RNase/DNase free water provided.

To ensure complete resuspension, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use.

Component – resuspend in water	Volume
Internal control primer/probe mix (BROWN)	165µl

3. Resuspend lyophilised DNA template in the template preparation buffer provided.

Component – resuspend in template preparation buffer	Volume
Internal control DNA template (BLUE)*	600µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

To use as an 'Internal extraction control' turn to **page 7**

To use as an 'Internal PCR control' turn to **page 8**

To use as an internal DNA extraction control

The internal control can be added either to the DNA lysis/extraction buffer or into the DNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal control DNA directly to the biological sample as this can lead to degradation and a loss in signal strength.

1. Add 4µl of internal control DNA template (**BLUE**) to each sample in DNA lysis/extraction buffer
2. Complete DNA extraction according to the manufacturer's protocol.
3. When using Primerdesign PrecisionPLUS 2X qPCR Master Mix or oasig 2X qPCR Master Mix, make up a mix containing all reagents according to the protocol below.

Component	1 Reaction
Target primer/probe mix	Xµl
Internal control primer/probe mix (BROWN)	1µl
PrecisionPLUS or oasig Master Mix	10µl
RNase/DNase free water (WHITE)	Xµl
Final volume	15µl

4. Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.
5. Prepare sample DNA templates for each of your samples (suggested concentration 5ng/µl) in RNase/DNase free water.
If the concentration of DNA is not known, then dilute your DNA samples 1:20 (10 µl of sample DNA and 190µl of water).
6. Pipette 5µl of diluted template into each well, according to your experimental plate set up.
The final volume in each well is 20µl.
7. Proceed to amplification protocol

To use as an internal PCR control

1. Prepare a 1:20 dilution of internal control DNA template.
2. When using Primerdesign PrecisionPLUS 2X qPCR Master Mix or oasis 2X qPCR Master Mix. Make up a mix containing all reagents according to the protocol below.

Component	1 Reaction
Target primer/probe mix	X μ l
Internal control DNA 1:20 dilution	1 μ l
Internal control primer/probe mix (BROWN)	1 μ l
PrecisionPLUS or oasis Master Mix	10 μ l
RNase/DNase free water (WHITE)	X μ l
Final volume	15μl

3. Pipette 15 μ l of this mix into each well according to your qPCR experimental plate set up.
4. Prepare DNA templates for each of your samples (suggested concentration 5ng/ μ l) in RNase/DNase free water.
If the concentration of DNA is not known, then dilute your DNA samples 1:20 (10 μ l of sample DNA and 190 μ l of water).
5. Pipette 5 μ l of diluted template into each well, according to your experimental plate set up.
For negative control wells use 5 μ l of RNase/DNase free water. The final volume in each well is 20 μ l
6. Proceed to amplification protocol

qPCR amplification protocol

Amplification conditions using Primerdesign PrecisionPLUS 2X qPCR Master Mix or oasig 2X qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 mins	95°C
X 40 cycles	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

* Fluorogenic data for the control DNA should be collected during this step through the channel specified in the introduction

If intending to use this kit with a FAST cycling protocol, please contact Primerdesign for further information, support@primerdesign.co.uk

Interpretation of results

When used according to the above protocols and assuming a 100% extraction efficiency, a Cq value of 28 is expected. However, this can vary significantly depending on the extraction efficiency, the quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28 ± 3 are within the normal range. When amplifying a target gene with a high copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.