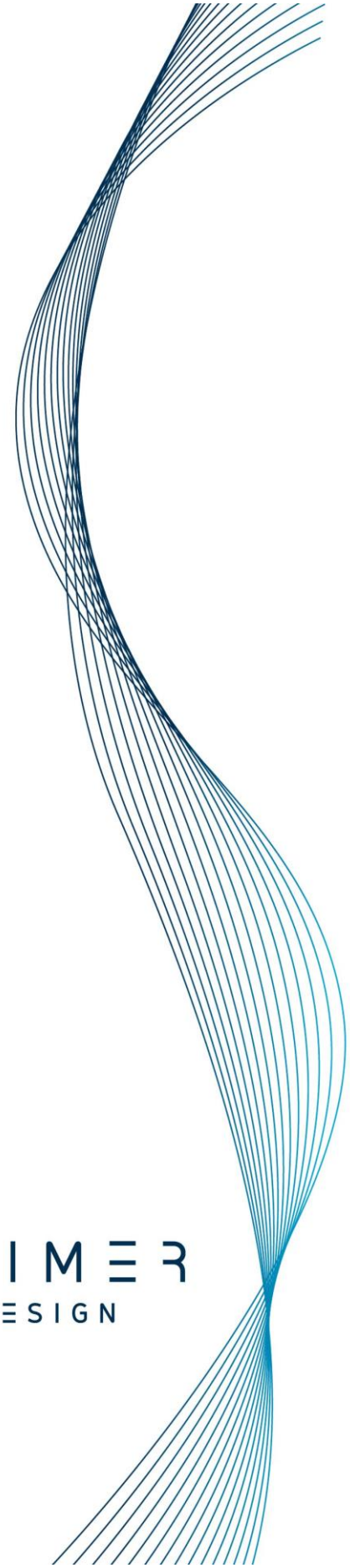
# Internal DNA extraction control

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



PRIMER DESIGN

# Contents

# 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 -20ºC 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.

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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.

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# 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.

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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 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 DNA 1:20 dilution | 1µl |
| Internal control primer/probe mix (**BROWN**) | 1µl |
| PrecisionPLUS or oasig Master Mix | 10µl |
| RNase/DNase free water (**WHITE**) | Xµl |
| **Final volume** | **15µl** |

3. **Pipette 15µl 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/µl) in RNase/DNase free water.**
   If the concentration of DNA is not known, then dilute your DNA samples 1:20 (10µl of sample DNA and 190µl of water).

5. **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

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.