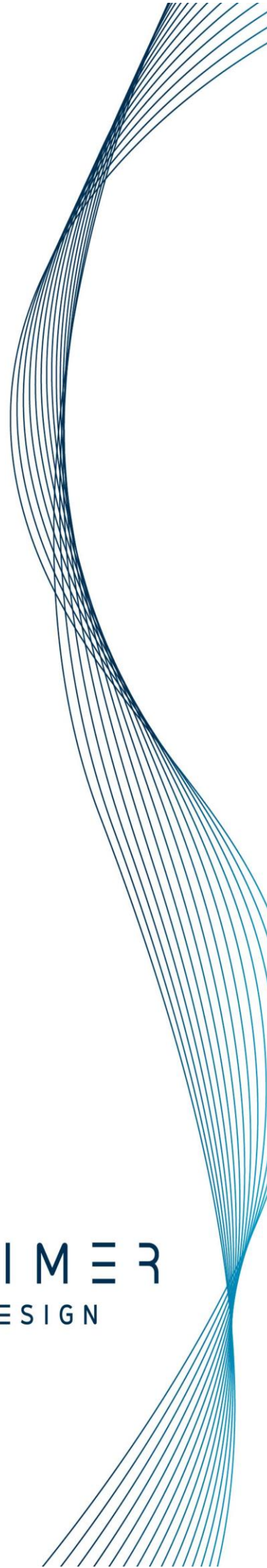


Reference gene detection assay

Instructions for detection and quantification of a
reference gene using SYBR[®] Green detection chemistry

PRIMER
DESIGN



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Introduction

This kit provides reagents for the target specific amplification of a reference gene using real-time PCR in conjunction with SYBR® Green detection chemistry. When resuspended, this kit provides primers that have been tested for priming specificity, and amplification efficiency at optimal concentrations. Real-time PCR is a very sensitive technology and it is not recommended to use more or less than the specified amount of primer in each reaction. However, final reaction volumes between 15µl and 50µl are often successful and may be tested at the user's discretion. Unfortunately, Primerdesign is not able to provide technical support for protocols other than those provided.

For accurate gene expression measurements, it is essential to normalise results from your quantitative real-time PCR experiments to a fixed reference.

- Normalising to a constitutively expressed reference gene is the most common method. Primerdesign provides researchers with a range of high quality real-time PCR assays for reference gene expression data.
- geNorm™ is a system for selecting optimal reference genes for any biological system (e.g. cell line, tissues sample).

Both can be found at www.primerdesign.co.uk

Kit contents

- Lyophilised primer set for a reference gene selected by the user (**BLUE**)
- RNase/DNase free water (**WHITE**)

Reagents and equipment to be supplied by user

- **Real-Time PCR instrument**
- **Master Mix or Master Mix components containing SYBR® Green**
This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign 2X qPCR Precision®PLUS or Precision®FAST Master Mix.
- **Pipettors and Tips**
- **Vortex and Centrifuge**
- **cDNA template**
The quality of cDNA will directly affect the quality of data generated using this kit. Primerdesign recommends the use of the Primerdesign Precision nanoScript™2 Reverse Transcription kit to generate cDNA from RNA.

Kit storage

This Primerdesign kit should be stored at -20°C on arrival. Freeze/thawing cycles should be kept to a minimum once resuspended. Under these conditions reagents are stable for six months from date of resuspension.

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.

SYBR® Green is a registered trademark of Molecular Probes.

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 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.

Resuspension 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 mix is in the base of the tube and is not spilt upon opening the tube.

2. Resuspend lyophilised primer 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.

Reaction number	Resuspension volume
300 rxn	330µl
600 rxn	660µl
900 rxn	990µl
1200 rxn	1320µl

There is a 10% over pipette in each tube.

3. For each qPCR reaction, including negative controls, make up a mix containing all qPCR reagents according to the protocol below:

Component	1 Reaction
Resuspended primer mix (BLUE)	1µl*
Primerdesign 2X qPCR PrecisionPLUS/PrecisionFAST Master Mix	10µl
RNase/DNase free water (WHITE)	4µl
Final volume	15µl

*working concentration of primers = 300nM in a 20µl reaction

4. Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.

5. Include the following negative control wells:

a) Include wells where the cDNA is replaced with RNase/DNase free water. Any amplification in this sample is indicative of cDNA cross contamination between wells, or contamination of one or more reagents.

B) Include wells where the equivalent concentration of RNA is added minus the reverse transcription step. Amplification of these wells may indicate genomic DNA contamination of your RNA sample. A DNase treatment step is highly recommended during RNA extraction to prevent this occurring.

6. Prepare cDNA for each of your samples (suggested concentration 5ng/μl) in RNase/DNase free water.

If the concentration of cDNA is not known, then dilute your RT reactions 1:10 (10μl of RT and 90μl of water)

7. Pipette 5μl of diluted cDNA into each well, according to your qPCR experimental plate set up.

The final volume in each well is 20μl.

qPCR amplification protocol

Amplification protocol for Primerdesign 2X qPCR PrecisionPLUS Master Mix.

	Step	Time	Temp
	Enzyme activation	2min	95°C
Cycling x40	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C
	Melt Curve**		

*Fluorogenic data should be collect during this step through the SYBR® Green channel.

**A post PCR run melt curve can be used to prove the specificity of the primers. See the manufacturer's instructions for your hardware platform

Amplification protocol for Primerdesign 2X qPCR PrecisionFAST Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95°C
Cycling x40	Denaturation	5s	95°C
	DATA COLLECTION*	20s	60°C
	Melt Curve**		

*Fluorogenic data should be collect during this step through the SYBR® Green channel.

**A post PCR run melt curve can be used to prove the specificity of the primers. See the manufacturer's instructions for your hardware platform