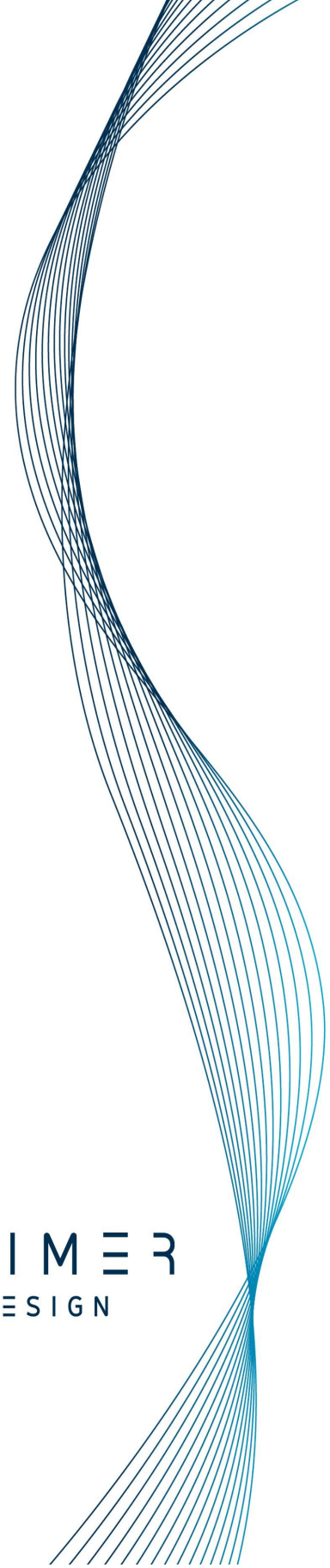
Genomic DNA quantification assay

Quantification of genomic DNA by real-time PCR



PRIMER DESIGN

Contents

Kit contents

- **Genomic DNA primer/probe mix (150 reactions, BROWN)**
- **Positive control genomic DNA template (for standard curve, RED)**
- **Internal extraction control primer/probe mix (150 reactions, BROWN)**
- **Internal extraction control DNA template (150 reactions, BLUE)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes.
- **Template preparation buffer (YELLOW)**
  for resuspension of internal extraction control DNA template, positive control template and standard curve preparation.

Reagents and equipment to be supplied by user

- **Real-Time PCR Instrument**
- **DNA extraction kit**
  This kit is recommended for use with genesig® EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.
- **Precision®PLUS, Precision®FAST or oasig™ 2X qPCR Master Mix**
  This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS, PrecisionFAST or oasig 2X qPCR Master Mix.
- **Pipettors and Tips**
- **Vortex and centrifuge**
- **Thin walled 0.2 ml PCR reaction tubes**

Kit storage and stability

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.
If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution, a fresh standard curve can be prepared from the positive control.

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, DNA integrity. An internal PCR control is supplied to test for non-specific PCR inhibitors. Always run at least one negative control with the samples. To prepare a negative control, replace the test 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 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.

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Principles of the test

Introduction

This kit provides reagents for the detection and quantification of genomic DNA for the species of interest. The primers detect a single copy region of non-transcribed DNA. The kit will not therefore detect cDNA. This removes false positive signals from this potential source of contamination.

The kit contains a number of additional components to avoid both false positive and false negative test results.

Real-time PCR

When resuspended, this kit provides primers that have been tested for priming specificity and amplification efficiency at optimal concentrations. qPCR is a very sensitive technology and it is not recommended to use more or less than the specified amount of primer and probe 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.

Positive control

For the purpose of DNA quantification, and as a positive control for the PCR set up, the kit contains positive control genomic DNA. This can be used to generate a standard curve of genomic DNA quantity / Cq value. Alternatively, the positive control can be used at a single dilution for a qualitative analysis of the samples. Each time the kit is used, at least one positive control reaction must be included on the run. A positive result indicates that the primers and probe for quantification of the target genomic DNA are working on that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false positive results. This can be achieved by handling this component in a Post-PCR environment.

Negative control

To confirm absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources or contamination should first be explored and removed.

Internal DNA extraction control

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 genomic DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR amplification of the control DNA also indicates that PCR inhibitors are not present at a high concentration.

A separate primer/probe mix are supplied with this kit to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the gDNA target sequence primers. Amplification of the control DNA does not interfere with detection of the target gDNA even when present at low copy number. The internal control is detected through the **VIC** channel and gives a CT value of 28+/-3.

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. The positive control template is a significant contamination risk and should therefore be pipetted after negative control and sample wells.

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 the kit components in RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component – resuspend in water	Volume
Pre-PCR pack	
gDNA primer/probe mix (**BROWN**)	165µl
Internal extraction control primer/probe mix (**BROWN**)	165µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive control template (**RED**) *	100µl
Pre-PCR heat sealed foil	
Internal extraction control DNA (**BLUE**)	600µl

* This component contains a high concentration of genomic DNA and is a significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

DNA extraction

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

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

1. **Add 4µl of internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer**

2. **Complete DNA extraction according to the manufacturer's protocols**

qPCR detection protocol

1. **Prepare the genomic DNA quantification mix according to table below:**

Component	1 reaction
PrecisionPLUS, PrecisionFAST or oasig Master Mix	10µl
gDNA primer/probe mix (**BROWN**)	1µl
Internal extraction control primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (WHITE)	3µl
Final volume	**15µl**

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

3. **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).

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. Preparation of 1:4 standard curve dilution series

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2 – 6.
2) Pipette 30µl of positive control template (**RED**) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 30µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard curve	DNA conc.
Tube 1 positive control (**RED**)	5000 pg/µl
Tube 2	1250 pg/µl
Tube 3	312 pg/µl
Tube 4	78 pg/µl
Tube 5	19 pg/µl
Tube 6	5 pg/µl

6. Pipette 5µl of standard curve dilution templates into each well, according to your experimental plate set up.

The final volume in each well is 20µl.

qPCR amplification protocol

Please select the correct cycling protocol for the master mix that you are using.

Amplification conditions using PrecisionPLUS or oasig 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95ºC
X 40 cycles	Denaturation	10s	95ºC
	DATA COLLECTION*	60s	60ºC

*Fluorogenic data should be collected during this step through the FAM and VIC channels.

Amplification conditions using PrecisionFAST 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95ºC
X 40 cycles	Denaturation	5s	95ºC
	DATA COLLECTION*	20s	60ºC

*Fluorogenic data should be collected during this step through the FAM and VIC channels.

Interpretation of results

For quantitative analysis, a standard curve must be plotted. The concentration of test samples can then be calculated by reference to the standard curve. Most modern hardware platforms will do this automatically when correctly configured. Please consult your manufacturer's handbook for instructions on this.

For qualitative analysis, the table below can be used to interpret the data

Genomic DNA	Internal control	Negative control	Positive control	Interpretation
+ve	+ve	-ve	+ve	+ve
+ve	-ve	-ve	+ve	+ve
-ve	+ve	-ve	+ve	-ve
-ve	-ve	-ve	-ve	Exp Fail
+ve	+ve	+ve	+ve	Exp Fail

Internal PCR control

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.