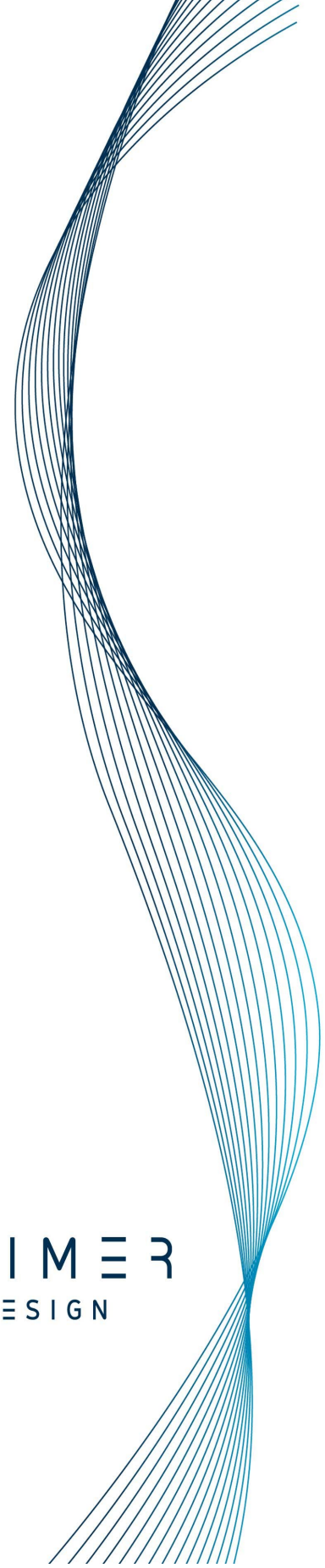
Genomic DNA detection assay

Detection of genomic DNA by real-time PCR



PRIMER DESIGN

Contents

Kit contents

- **Genomic DNA primer/probe mix (150 reactions, BROWN)**
- **RNase/DNase free water (WHITE)**

Reagents and equipment to be supplied by user

- **Real-Time PCR Instrument**
- **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 re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

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 test sample with RNase/DNase free water.

Licensing agreement and limitations of use

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Primerdesign satisfaction guarantee

Primerdesign takes pride in the quality of all of 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 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.

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.

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.

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 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	Volume
gDNA primer/probe mix (**BROWN**)	165µl

3. **Prepare the gDNA detection mix according to the table below:**

Component	1 reaction
PrecisionPLUS or PrecisionFAST or oasig Master Mix	10µl
gDNA primer/probe mix (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Final volume	**15µl**

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

5. **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).

6. **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.

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 channel.

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 channel.