Primerdesign™

oasig™ lyophilised 2X qPCR Master Mix

Instructions for use of Primerdesign oasig lyophilised Master Mix

oasig lyophilised 2X qPCR Master Mix Handbook HB04.03.05
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Introduction

Primerdesign oasig lyophilised 2X qPCR Master Mix is optimised for use in real-time PCR. The product is stable at ambient temperatures for at least 18 months and can be conveniently shipped and stored at room temperature. The master mix contains a thermo-stable TAQ Polymerase as well as buffer, dNTPs, $MgCl_2$ and stabilisers at concentrations optimised for the enzyme. The master mix has been freeze-dried to produce a room temperature stable preparation.

The kit includes the lyophilised master mix, resuspension buffer and a tube of ROX dye which can be added as required when the master mix is to be used on hardware platforms that use ROX as a passive reference dye.

The resuspended solution requires only the addition of your cDNA, primers and probe to be PCR ready.

The performance of oasig lyophilised 2X qPCR Master Mix is as good or better than leading brands. For details see www.primerdesign.co.uk

The perfect partner for genesig kits

Primerdesign oasig lyophilised 2X qPCR Master Mix is designed for use with our range of genesig qPCR pathogen detection kits. Together they represent the ultimate solution for convenient logistics as well as high quality qPCR performance.

Kit contents

- **3 x lyophilised Master Mix (50 reactions per glass ampule)**
- **1 x lyophilised ROX (BROWN)**
- **4 x Resuspension buffer (BLUE)**

Kit storage

The Primerdesign oasig lyophilised 2X qPCR Master Mix should be stored at ambient temperature on arrival. The kit is stable for at least 18 months at ambient temperature. Once resuspended in the provided buffer the kit should be stored at -20°C. Repeated freeze/thawing will not compromise the performance of the product. Under these conditions reagents are stable for six months from date of resuspension.

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

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 Ltd 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 routine quality assurance programme, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.

Resuspension protocol

1. **For each glass ampule resuspend lyophilised Master Mix in 525µl of resuspension buffer**
   Do not replace the resuspension buffer with water or any other buffer.
   The master mix is then ready to use as a 2X qPCR master mix

2. **Add ROX if required**
   ROX is required for platforms that use ROX as a passive reference guide. Use table 1 below to see if ROX addition is required for your hardware platform. If ROX is required then follow the instructions below.

   - Resuspend the lyophilised ROX (**BROWN**) in the correct volume of resuspension buffer (**BLUE**) according to table 1 below.
   - Add resuspended ROX to each ampule at the correct level.

Table 1. ROX addition

Real-time PCR platform	ROX resuspension volume	ROX addition per ampule
Applied Biosystems 7700, 7000, and 7900, 7300 StepOne, StepOnePLUS and ViiA7 platforms, Roche capillary Lightcyclers.	100µl	20µl
All Stratagene platforms	200µl	15µl
Applied Biosystems 7500 platform	700µl	10µl
All Other machines	NOT REQUIRED	NOT REQUIRED

qPCR detection protocol

- **When using Primerdesign genesig pathogen detection kits.**

For each 20µl qPCR reaction add the following to each reaction tube

Components	1 Reaction
oasig lyophilised 2X qPCR Master Mix	10 µl
Primer/probe mix	1 µl
Template (25ng)	5 µl
RNase/DNase free water	4 µl
Final volume	**20 µl**

- **Suggested use with user supplied primers and probe.**

For each 20µl qPCR reaction add the following to each reaction tube

Components	1 Reaction
oasig lyophilised 2X qPCR Master Mix	10 µl
Forward primer (6pmol*)	x µl
Reverse primer (6pmol*)	x µl
Probe (3pmols)	x µl
Template (25ng)	x µl
RNase/DNase free water (up to Final volume)	x µl
Final volume	**20 µl**

*6pmols of primer gives a working concentration of 300nM in a 20µl reaction

qPCR amplification protocol

- **For use with genesig pathogen detection kits**

	Step	Time	Temp
	Enzyme Activation	2 min	95ºC
Cycling x50	Denaturation	10 s	95ºC
	DATA COLLECTION*	60 s	60ºC

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