

Primer Design Ltd

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oasig[®]PLUS OneStep Lyophilised 2X RT-qPCR Master Mix

Instructions for use of Primerdesign oasig[®]PLUS OneStep Lyophilised
Master Mix

150 tests

PRIMER
DESIGN

For general laboratory and research use only

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Introduction

Primerdesign oasisig®PLUS OneStep Lyophilised 2X RT-qPCR Master Mix is an optimised complete Master Mix product for use in OneStep real-time PCR. Use of a OneStep qPCR reaction, including a Reverse Transcription enzyme, reduces handling errors, and greatly shortens the time taken to obtain results. oasisig®PLUS OneStep Master Mix utilises a tightly controlled temperature-dependent Reverse Transcriptase enzyme. This improves multiplexing performance with higher consistency by minimizing unwanted non-specific priming and extension during room temperature set-up. The master mix contains a superior Reverse Transcriptase (as described above) and a hot start Taq DNA Polymerase as well as buffer, dNTPs, MgCl₂ and stabilisers at concentrations optimised for the enzymes and qPCR reaction. Once resuspended, only the template RNA and primer/probe mix are required to complete the experimental set up for a perfect multiplex reaction. For greater flexibility, the master mix is freeze-dried to produce a room temperature stable reagent, which can be conveniently shipped at ambient but should be stored at -20°C on arrival.

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

If you require further information or have a specific question about this master mix then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

The perfect partner for genesig® kits

Primerdesign oasisig®PLUS OneStep Lyophilised 2X RT-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 oasig®PLUS OneStep Lyophilised Master Mix (50 rxns per glass vial, GOLD)**
- **1 x oasig® Lyophilised ROX (BROWN)**
ROX passive reference dye that if required can be added to oasig®PLUS OneStep Master Mix
- **4 x oasig® resuspension buffer (BLUE)**
for resuspension of the oasig®PLUS OneStep Master Mix (and Lyophilised ROX, if required)

Kit storage

The Primerdesign oasig®PLUS OneStep Lyophilised 2X RT-qPCR Master Mix is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the oasig®PLUS OneStep Master Mix have been resuspended, it should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided as it may compromise the performance of the Master Mix. Under these conditions reagents are stable for six months from date of resuspension

Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of RNA sample material can be used (e.g. Viral RNA, cell culture derived RNA, Biopsy derived RNA etc). Please ensure the samples are suitable in terms of purity, concentration, and RNA 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

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.

Resuspension protocol

1. For each glass vial resuspend oasisig®PLUS OneStep 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 RT-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 vial at the specific volume.

Table 1. The recommended ROX dye addition levels for various instruments

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

OneStep RT-qPCR detection protocol

- When using Primerdesign genesis[®] pathogen detection kits.

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

Components	1 Reaction
oasig [®] PLUS OneStep 2X RT-qPCR Master Mix	10 µl
Primer/Probe mix	1 µl
Template RNA	x µl
RNase/DNase free water	x µl
Final volume	20 µl

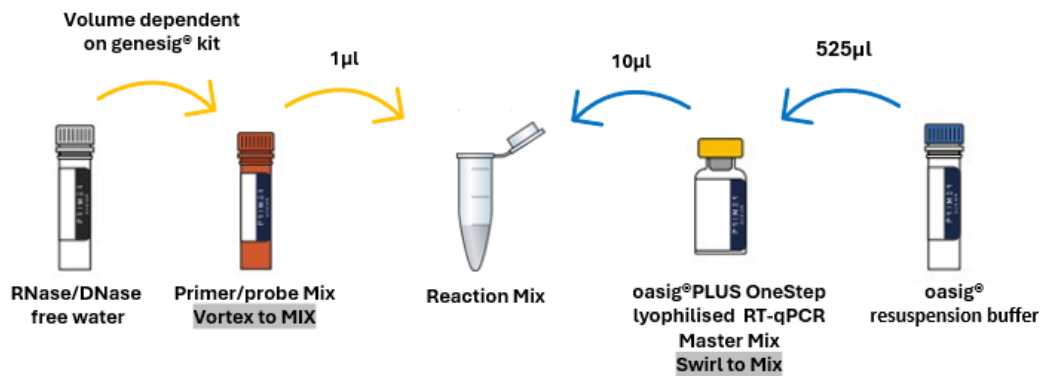


Figure 1. Overview of the reaction mix set-up.

- Suggested use with user supplied primers and probe.

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

Components	1 Reaction
oasig [®] PLUS OneStep 2X RT-qPCR Master Mix	10 µl
Forward primer (3pmols*)	x µl
Reverse primer (3pmols*)	x µl
Probe (3pmols)	x µl
Template RNA	x µl
RNase/DNase free water (up to Final volume)	x µl
Final volume	20 µl

*3pmols of primer gives a working concentration of 150nM in a 20µl reaction

OneStep RT-qPCR amplification protocol

- For use with genesig® pathogen detection kits

	Step	Time	Temp
	Reverse Transcription	10 min	55°C
	Enzyme Activation	2 min	95°C
Cycling x50	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

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