Primer Design Ltd

oasig® lyophilised OneStep 2X RT-qPCR Master Mix

Instructions for use of Primerdesign oasig® lyophilised OneStepMaster Mix

For general laboratory and research use only

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Introduction

Primerdesign oasig® lyophilised OneStep 2X RT-qPCR Master Mix is an optimised complete system for use in OneStep real-time PCR. Removal of a separate Reverse Transcription step, reduces handling errors, and greatly reduces the time taken to obtain results. The product is stable at ambient temperatures so it can be conveniently shipped but should be stored at -20ºC on arrival. The master mix contains a thermo-stable Taq DNA Polymerase and MMLV Reverse Transcriptase as well as buffer, dNTPs, $MgCl_2$ and stabilisers at concentrations optimised for the enzymes. Once resuspended, only the template RNA and primer and probe mix are required to complete the experimental set up for a perfect single tube reaction. The master mix has been freeze-dried to produce a room temperature stable preparation.

The kit includes the oasig® lyophilised OneStep master mix, oasig® 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 performance of oasig® lyophilised OneStep 2X RT-qPCR Master Mix is as good as or better than leading brands. For details see www.primerdesign.co.uk

If you require further information or have a specific question about this profile 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 oasig® lyophilised OneStep 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® lyophilised OneStep Master Mix (50 rxs per glass ampule, RED)**
- **1 x oasig® lyophilised ROX (BROWN)**
  ROX passive reference dye that if required can be added to oasig® lyophilized OneStep Master Mix
- **4 x oasig® resuspension buffer (BLUE)**
  for resuspension of the oasig® lyophilised OneStep Master Mix (and lyophilized ROX, if required)

Kit storage

The Primerdesign oasig® lyophilised OneStep 2X RT-qPCR Master Mix is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the oasig® lyophilised 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

Primer Design Ltd 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 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

1. **For each glass ampule resuspend oasig® lyophilised OneStep 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 ampule at the correct level.

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

Real-time PCR platform	ROX resuspension volume	ROX addition per ampule
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 genesig® pathogen detection kits.**

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

Components	1 Reaction
oasig® lyophilised 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**

- **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® lyophilised 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	55ºC
	Enzyme Activation	2 min	95ºC
Cycling x50	Denaturation	10s	95ºC
	DATA COLLECTION*	60s	60ºC

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