

oasig[™] PLEX Lyophilised OneStep 2X RT-qPCR Master Mix

Instructions for use of Primerdesign oasigPLEX
Lyophilised OneStep 2X RT-qPCR Master Mix

**PRIMER
DESIGN**

Contents

Introduction	3
Kit contents	4
Kit storage	4
Suitable sample material	4
Licensing agreement and limitations of use	4
Primerdesign satisfaction guarantee	4
Quality control	4
Resuspension protocol	5
OneStep RT-qPCR detection protocol	6
OneStep RT-qPCR amplification protocol	7

Introduction

Primerdesign oasigPLEX Lyophilised OneStep 2X qPCR Master Mix is a freeze-dried speciality master mix optimised for use in multiplex RT-qPCR. oasigPLEX OneStep Master Mix contains optimised enzyme levels and our proprietary enzyme-buffer system to maximise performance of multiplex assays. Included in our oasigPLEX OneStep RT-qPCR Master Mix is ampliSOLVE, an innovative solution that will enhance qPCR efficiency through artefact removal. The function of ampliSOLVE is to remove amplicon contamination and reduce the possibility of primer dimer formation. Resulting in an enhanced efficiency and performance of the RT-qPCR reaction.

The RT-qPCR master mix is designed for rapid cycling protocols and contains an antibody mediated Hot-Start mechanism which releases more active enzyme and requires a much shorter activation time. Removal of a separate reverse transcription step, reduces handling errors, and greatly reduces the time taken to obtain results. The freeze-dried 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 and MMLV as well as buffer, dNTPs, MgCl₂ and stabilisers at concentrations optimised for the enzyme.

The kit includes the Lyophilised master mix, resuspension buffer and a tube of ROX dye which can be added as required, if the master mix is to be used on hardware platforms that use ROX as a passive reference dye. Please note this should not be used if ROX is also being used as a reporter dye in a multiplex RT-qPCR reaction.

The oasigPLEX OneStep Master Mix is a truly unique product combining the ultimate solution for multiplex RT-qPCR with simple, convenient storage and logistics.

Kit contents

- 3 x oasigPLEX Lyophilised OneStep Master Mix (50 reactions per glass ampoule)
- 1 x Lyophilised ROX (**BROWN**)
- 4 x resuspension buffer (**BLUE**)

Kit storage

The Primerdesign oasigPLEX Lyophilised OneStep 2X RT-qPCR Master Mix kit should be stored at room temperature on arrival. The kit is stable for at least 18 months at room temperature.

Once resuspended in the provided buffer the kit should be stored at -20°C and unnecessary repeated freeze/thawing should be avoided. Under these conditions, reagents are stable for six months from date of resuspension.

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 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 ampoule resuspend oasigPLEX 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 ampoule at the correct level.

Table 1. ROX addition

Real-Time PCR platform	ROX resuspension volume	ROX addition per ampoule
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

Please note, ROX should not be added to the master mix if using a ROX fluorophore as a reporter dye in the multiplex assay. In this case, if using a machine that requires ROX as a passive reference dye, select “none” as passive reference dye option.

OneStep RT-qPCR detection protocol

- **When using user supplied multiplex assay:**

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

Components	1 Reaction
oasigPLEX Lyophilised OneStep 2X RT-qPCR Master Mix	10µl
Multiplex primer/probe mix*	xµl
Template RNA	xµl
RNase/DNase free water (up to final volume)	xµl
Final volume	20µl

* Make a multiplex primer/probe mix, containing all required primers and probes, at desired concentration.

OneStep RT-qPCR amplification protocol

- For use with user supplied multiplex assay

	Step	Time	Temp
	Reverse transcription	10min	55°C
	Enzyme activation	2min	95°C
Cycling x 50	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

*Fluorogenic data should be collected during this step through the relevant channels.