PCRMax™

Lyophilised OneStep 2X RT-qPCR Master Mix

Instructions for use of Lyophilised OneStep Master Mix

Lyophilised OneStep 2X RT-qPCR Master Mix Handbook HB04.04.05
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Introduction

Our 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 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, $MgCl_2$ 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 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.

Kit contents

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

Kit storage

The Lyophilised OneStep 2X RT-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 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 this kit does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

Resuspension protocol

1. **For each glass ampule resuspend 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. 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

OneStep RT-qPCR detection protocol

- **When using PCRmax qPCR test kits.**

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

Components	1 Reaction
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
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 PCRmax qPCR test kits**

	Step	Time	Temp
	Reverse Transcription	10 min	55ºC
	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 channel.