



Techne lyophilised One Step qRT-PCR MasterMix

Instructions for use of Techne Prime Pro lyophilised One Step MasterMix

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Introduction

Techne Prime Pro qRT-PCR MasterMix is an optimised complete system for use in ONE STEP 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 MasterMix contains a thermo-stable TAQ Polymerase and MMLV as well as buffer, MgCl₂ and stabilisers at concentrations optimised for the enzymes. Once reconstituted, only the template RNA and primer and probe mix are required to complete the experimental set up for a perfect single tube reaction. The MasterMix has been freeze-dried to produce a room temperature stable preparation.

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

The performance of Techne lyophilised MasterMix is as good as or better than leading brands.

Kit Contents

- 3 x Lyophilised MasterMix (50 reactions per glass ampule)
- 1 x Lyophilised ROX (**BROWN**)
- 4 x Re-suspension buffer (**BLUE**)

Kit Storage

The Techne Lyophilised One Step MasterMix 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 Techne kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

Techne Satisfaction Guarantee

Techne 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, Techne will replace the item free of charge.

Quality Control

As part of our routine quality assurance programme, all Techne products are monitored to ensure the highest levels of performance and reliability.

Bench-side Protocol

1. For each glass ampule; Re-suspend lyophilised One Step MasterMix in 525µl of re-suspension buffer

Do not replace the re-suspension buffer with water or any other buffer.

The MasterMix is then ready to use as a 2X qPCR MasterMix.

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.

- Re-suspend the Lyophilised ROX (**BROWN**) in the correct volume of re-suspension buffer(**BLUE**) according to table 1 below.
- Add re-suspended ROX to each ampule at the correct level.

Table 1. ROX addition

Real time PCR platform	ROX re-suspension 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

Reaction setup

- **When using Techne pathogen detection kits.**

For each 20µl real-time PCR reaction add the following to each reaction tube

Components	1 Reaction
Techne Lyophilised One Step MasterMix	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 real-time PCR reaction add the following to each reaction tube

Components	1 Reaction
Techne Lyophilised One Step MasterMix	10 µl
Primers (3pmols Forward and Reverse)	x µl
Probe (3pmols)	x µl
Template RNA	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

Amplification Protocol

- For use with Double Dye (TaqMan® gene detection chemistry)

	Step	Time	Temp
	Reverse Transcription	10 min	42°C
	Enzyme Activation - Hot Start	2 min	95°C
Cycling x50	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

*Fluorogenic data should be collected during this step according to the detection kit.

- For use with SYBR® Green detection chemistry

	Step	Time	Temp
	Reverse Transcription	10 min	42°C
	Enzyme activation - Hot Start	2 min	95°C
Cycling x50	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C
	Melt Curve**		

*Fluorogenic data should be collected during this step through the SYBR® Green channel.

**A post PCR run melt curve can be used to prove the specificity of the primers. See the manufactures instructions for your hardware platform