

WizPure™ qRT-PCR Master (EVA)

RUO For Research Use Only

REF W2721

DESCRIPTION

WizPure™ qRT-PCR Master (EVA) is an optimised ready-to-use solution for one-step quantitative RT-PCR assays, incorporating EvaGreen dye. It comprises all the components necessary to perform qRT-PCR: WizScript™ III RTase, antibody-mediated hot-start Taq DNA Polymerase, dNTPs, MgCl₂, EvaGreen dye, enhancer and stabilizer. The user simply needs to add water, template and primers. An enhanced buffer allows for RT reaction temperatures up to 50°C. This can improve detection of more difficult targets as higher RT temperatures reduce nonspecific priming and facilitate melting of RNA secondary structures. The kit includes the components necessary for performing RT-PCR amplification, and have been successfully used to DNA amplify and detect a variety of RNA targets. The kit provides a one-step, simple, robust, inexpensive assay for detection and quantitative analysis of gene expression directly from cells or RNA with intercalator format.

KIT CONTENTS

Contents	W2721	W2721-5
WizPure™ qRT-PCR Master (EVA)	1 ml	5 X 1 ml

APPLICATIONS

- One-step Real-Time RT-PCR
- Gene expression profiling
- Gene knockdown verification
- Array Validation

STORAGE CONDITIONS

Upon receipt, store all components at -20°C. Store the Master mix at 4°C after thawing for up to 6 months, depending on the expiration date, without showing any reduction in performance.

USE OF THE ROX REFERENCE DYE

(High ROX)

- ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
 - Amount per 50 µl reaction: 1.0 µl (0.6-1.0 µl)
 - Final ROX Concentration: 500nM (300-500nM)

(Low ROX)

- ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene Mx3000, Mx3005P and Mx4000:
 - Amount per 50 µl reaction: 0.1 µl (0.06-0.1 µl)
 - Final ROX Concentration: 50nM (30-50nM)

QUALITY CONTROL ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPure™ qRT-PCR Master (EVA) kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage. See pre-protocol considerations for details.

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the qRT-PCR Master (EVA) mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on

ice immediately after thawing.

2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Component	20 µl reaction	Final Conc.
qRT-PCR Master (EVA)	10.0 µl	1X
10µM Forward Primer	0.2~2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2~2.0 µl	0.1~1.0 µM
Template RNA	Variable	≤ 500 ng/reaction
Water, RNase-Free	up to 20 µl	NA

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM for specificity.

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
(Optional) Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
5. Transfer tubes into a Real-time PCR instrument and run as following table.

Step	Temp (°C)	Time	Cycle
Reverse Transcription	42 ~ 55	10 ~ 30 min.	1
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	30 ~ 45
Anneal (Detection)	55~68	10 ~ 60 sec.	
Melting curve analysis	65~95	2~5 sec./step	1

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

NOTE: Shorter annealing step time (<10sec) can be used for amplicon <100bp.

ORDERING INFORMATION

Product	Cat No.	Package
WizPure™ qRT-PCR Master (EVA)	W2721	100 rxn
	W2721-5	500 rxn

Technical Support



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