WizPure™ qPCR Master (EVA)

- W1721 **100 rxn** -
- W1721-8 **800 rxn** -
- W1721R **100 rxn** ROX
- W1721R-5 **500 rxn** ROX

Description

WizPure™ qPCR Master (EVA) is an optimized ready-to-use solution for real-time quantitative PCR assays, incorporating EvaGreen dye. It comprises all the components necessary to perform qPCR: antibody-mediated hot-start Taq DNA Polymerase, ultrapure dNTPs, MgCl2, Eva Green, enhancer and stabilizer.

The user simply needs to add water, template and primers. Hot start DNA Polymerase is activated by a 5 minutes incubation step at 95°C. This prevents extension of nonspecifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

The kit includes the components necessary for performing PCR amplification, and have been successfully used to amplify and detect a variety of DNA targets such as genomic DNA, cDNA and plasmid DNA.

Kit Contents

Contents	W1721	W1721-8	W1721R	W1721R-5
qPCR Master (EVA)	1 ml	8 X 1 ml	1 ml	5 X 1 ml
ROX Dye (50X)	-	-	50 µl	250 µl

Applications

- Real-time PCR
- Detection and quantification of DNA and cDNA targets
- Gene expression profiling & knockdown verification
- Microbial detection
- Viral load determination
- Array validation
- SNP genotyping

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.

Note

Do not contaminate the WizPure™ qPCR Master (EVA) mix with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

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:

PCR sensitivity and reproducibility assay

Sensitivity and reproducibility in real-time PCR are tested in parallel reactions containing 10-fold dilutions of nucleic acid template.

Quality Authorized by : Jamie Ahn

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 qPCR 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:

Reaction Conditions

Component	20 µl reaction	Final Conc.
qPCR Master (EVA)	10.0 µl	1X
ROX Dye (50X)* (optional)	0.4 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 DNA	Variable	≤ 500 ng/reaction
Water, RNase-Free	up to 20 µl	NA

* Please note "Use of the ROX Reference Dye"

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

NOTE: Recommended amount of template per PCR reaction:
- < 50 ng plasmid or
- < 500~1000ng genomic DNA or
- 2µl of a 100µl single plaque eluate or
- one single bacterial colony

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.

PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	25 ~ 40
Anneal	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.

RUO Research Use Only

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