WizPure™ qPCR Master-UDG (PROBE)

(For preventing carryover-contamination)

- W1702 **100 rxn** -
- W1702-8 **800 rxn** -
- W1702R **100 rxn** ROX
- W1702R-5 **500 rxn** ROX

Description

WizPure™ qPCR Master-UDG (PROBE) is ready-to-use 2X reagent ideal for most quantitative Real-time PCR applications. The master mix is recommended for use with Labeled Fluorescent Probes, e.g. for 5'-Nuclease Assays or Hybridization probes. The qPCR Master-UDG (PROBE) contains and antibody-mediated hot-start Taq DNA Polymerase, MgCl2, dATP, dCTP, dGTP, dUTP, Uracil DNA Glycosylase (UDG), enhancer and stabilizer.

UDG and dUTP are included in the mixture to prevent the reamplification of carryover PCR products between reactions. dUTP in the mix ensures that any amplified DNA will contain uracil. UDG removes uracil residues from single- or double-stranded DNA, preventing dU-containing DNA from serving as template in future PCRs.

Kit Contents

Contents	W1702	W1702-8	W1702R	W1702R-5
qPCR Master-UDG (PROBE)	1 ml	8 X 1 ml	1 ml	5 X 1 ml
ROX Dye (50X)	-	-	50 µl	250 µl

Applications

- Real-time 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)

Preventing Template Carryover-Contamination

Due to the high sensitivity of PCR it is a risk that reaction may be contaminated with the products of previous runs. To minimize this risk, both dUTP and UDG is included in the qPCR Master-UDG (PROBE) mix mix to prevent PCR products from becoming source of contamination.

Quality Control Analysis

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-UDG (PROBE) 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-UDG (PROBE)	10.0 µl	1X
ROX Dye (50X)* (optional)	0.4 µl (0.04µl)	1X (0.1X)
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
Fluorescence Probe	Variable	0.1~1.0 µM
Template DNA	Variable	
Water, RNase-Free	up to 20 µl	

* 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
Carryover prevention	50	2 min.	1
Initial Denaturation	95	10 min.	1
Denature	95	10 ~ 30 sec.	25 ~ 40
Anneal	55~68	10 ~ 60 sec.	

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.

RUO Research Use Only

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