CrystalMix™ qPCR-UDG (PROBE)

RUO For Research Use Only

REF W8702

DESCRIPTION

CrystalMix™ qPCR-UDG (PROBE) kit combines all the reagents necessary for successful routine real-time PCR in a convenient individually aliquot in an 8-strip qPCR tube. CrystalMix™ qPCR-UDG (PROBE) kit is an economical, highly efficient, ready-to-use, and room temperature stable format. There is no need for freezing, thawing steps, or pipetting on ice, so minimized the risk of human errors and contaminations.

CrystalMix™ qPCR-UDG (PROBE) kit contains antibody-mediated hot-start Taq DNA Polymerase, PCR buffer, MgCl2, and dATP, dCTP, dGTP, dUTP, and Uracil DNA Glycosylase (UDG). 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 a template in future PCRs.

APPLICATIONS

- High through-put Real-time PCR
- Gene expression profiling
- Gene knockdown verification
- Array validation
- Routine diagnostic PCR requiring high reproducibility
- Point-of-care Molecular diagnostics

STORAGE CONDITIONS

- Store at below 25°C in the airtight pouch with the desiccant.
- Once opened, completely reseal the pouch with zipper.
- In high humidity environments, store unopened and resealed pouches in a desiccator to maximize product lifetime.
- Do not use once the cone-shape mix shrinks as dot-form. It damaged by re-hydration.

USE OF THE ROX REFERENCE DYE

(High ROX)

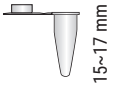
- ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
  - Amount per 50 µl reaction: 1.0 µl (0.6-1.0 µl)
  - Final ROX Concentration: 500nM (300-500nM)

(Low ROX)

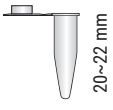
- ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene Mx3000, Mx3005P and Mx4000 :
  - Amount per 50 µl reaction: 0.1 µl (0.06-0.1 µl)
  - Final ROX Concentration: 50nM (30-50nM)

REAL-TIME PCR TUBE SELECTION GUIDE

- Low-profile (0.1ml) tube
  - ABI* : 7500 Fast, 7900HT Fast, StepOne, StepOnePlus, QuantiStudio Fast,
  - Bio-Rad : CFX96
  - Roche : LightCycler 480, LightCycler 96



- High-profile (0.2ml) tube
  - ABI : 7300, 7500, 7500HT, QuantiStudio, ViiA7
  - Agilent : Mx3000P, 3005P, Mx4000



* ABI : Applied Biosystems (ThermoFisher Scientific)

QUALITY CONTROL ANALYSIS:

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot CrystalMix™ qPCR-UDG (PROBE) 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.

Please read through the entire protocol before starting.

Use the required number of tubes and immediately put the remaining tubes in the pouch and seal with the zipper.

1. Prepare the reaction mixture as the following table.

Component	20 µl reaction	Final Conc.
qPCR-UDG (PROBE) tube	1 tube	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, DNase-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
- < 500~1000 ng genomic DNA or
- 2 µl of a 100 µl single plaque eluate or
- one single bacterial colony

2. 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 a heated lid on a thermal cycler.

3. Transfer tubes into a Real-time PCR instrument and run as the following table.

Step	Temp (°C)	Time	Cycle
Carryover prevention	50	2 min.	1
Initial Denaturation	95	10 min.	1
Denature	95	10 ~ 30 sec.	30 ~ 45
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.

ORDERING INFORMATION

Product	Cat No.	Package	Tube size
CrystalMix™ qPCR-UDG (PROBE)	W8702L	96 rxn	0.1 ml
	W8702H	96 rxn	0.2 ml

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