CrystalMix™ qPCR (PROBE)

- Real-time PCR tablet mix in a 8-strip qPCR tube

- W8701L **96 rxn** (0.1ml tube)
- W8701H **96 rxn** (0.2ml tube)

Description

CrystalMix™ qPCR (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 (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 (PROBE) kit is an optimized, ready-to-use PCR mixture of antibody-mediated hot-start Taq DNA Polymerase, PCR buffer, MgCl2 and dNTP's, except DNA template and primers.

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

Sensitivity and reproducibility in real-time PCR are tested in parallel reactions containing 10-fold dilutions of the 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.
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 qPCR (PROBE) tube on the PCR tube rack.
2. Add the reaction component to the qPCR (PROBE) tube as the following table shows recommended component volumes:

Reaction Conditions

Component	20 µl reaction	Final Conc.
qPCR (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	
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

3. 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.
4. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

PCR Conditions

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 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.

RUO Research Use Only

wizbiosolutions THE INNOVATION OF GENOMICS
TEL : +82 (0)70 7605 5066 sales@wizbiosolution.com
FAX : +82 (0)31 624 3066 www.wizbiosolution.com