

DESCRIPTION

CrystalMix™ qRT-PCR (PROBE) kit combines all the reagents necessary for successful one-step real-time RT-PCR in a convenient individually aliquot and freeze-dried in 8-strip qPCR tube. CrystalMix™ qRT-PCR (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™ qRT-PCR (PROBE) kit is an optimized, ready-to-use RT-PCR mixture of antibody-mediated hot-start Taq DNA Polymerase, WizScript™ RTase, Reaction buffer, MgCl₂ and dNTP's, except DNA template and primers. An enhanced buffer allows for RT reaction temperatures up to 50°C. This can improve the detection of more difficult targets as higher RT temperatures reduce nonspecific priming and facilitate the melting of RNA secondary structures.

APPLICATIONS

- High through-put Real-time RT-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)

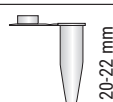
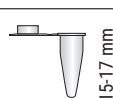
- ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
 - Amount per 50 µL reaction: 1.0 µL (0.6-1.0 µL)
 - Final ROX Concentration: 500nM (300-500nM)

(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 (0.1 mL) PCR tube**
 - ABI* : 7500 Fast, 7900HT Fast, StepOne, StepOnePlus, QuantStudio Fast,
 - Bio-Rad : CFX96
 - Roche : LightCycler 480, LightCycler 96
- **High-profile (0.2 mL) PCR tube**
 - ABI : 7300, 7500, 7500HT, QuantStudio, 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™ qRT-PCR (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 qRT-PCR (PROBE) tube on a PCR tube rack.
2. Add the reaction component to the qRT-PCR (PROBE) tube as the following table shows recommended component volumes:

Component	20 µL reaction	Final Conc.
qRT-PCR (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 RNA	≤ 5 µL	≤ 500 ng/reaction
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.

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:

Step	Temp (°C)	Time	Cycle
cDNA synthesis	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.	

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™ qRT-PCR (PROBE)	W8721L	96 rxn	Low-profile tube
	W8721H	96 rxn	High-profile tube

Technical Support



www.wizbiosolution.com

support@wizbiosolution.com

+82 70 7605 5066



Wizbiosolutions Inc.

#1103, 1405, 1406, A-dong, 14, Sagimakgol-ro 45beon-gil, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea, B237-B242, 14, Galmachi-ro 288beon-gil, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea