# Q-Starter Pack™ qPCR (PROBE)

If you choose the appropriate type, please order by that number.

- W1201 **1 ml X 8**
- W1202 **1 ml X 8**

## Description

Q-Starter Pack™ kit is ideal for set-up of Real-time PCR setup. The Q-Starter Pack™ qPCR (PROBE) kit is composed 8 different type of qPCR master mix for use with labeled fluorescent probes, e.g. for 5′-Nuclease Assays or Hybridization probes. All of qPCR Master mix includes the components necessary for performing Real-time 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

| Cat No. | Type | Contents |
|---|---|---|
| W1201 | Q-Starter Pack™ qPCR (PROBE) | 1 ml X (No. 1~8) |
| W1202 | Q-Starter Pack™ qPCR-UDG (PROBE) | 1 ml X (No. 1~8) |

## Applications

- Real-time PCR set-up

## 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 Q-Starter Pack™ qPCR (PROBE) kit 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

ROX reference dye is not included in this kit and may be added to compensate for non-PCR related variations in fluorescence. Addition of the reference dye is optional. Optimizing the ROX dye concentration within the qPCR reaction is an important aspect of setup. Too much ROX in the qPCR reaction will reduce background but also makes a low target signal difficult to distinguish from background.

## 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 (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. |
|---|---|---|
| qRT-PCR Master | 10.0 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 |
| Fluorescence Probe | Variable | 0.1~1.0 µM |
| Template RNA | Variable | ≤ 500 ng/reaction |
| Water, RNase-Free | up to 20 µl | NA |

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. | 30 ~ 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.

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