# TAQMAN PROBE FAST qPCR MASTER MIX FS-T-1072F

## Description

TaqMan Probe Fast qPCR Master Mix 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 TaqMan Probe Fast qPCR Master Mix is an optimized, ready-to-use PCR mixture of Hot-start Taq DNA Polymerase, PCR buffer, Magnesium and dNTPs, except DNA template and primers. The kit includes the components necessary for performing 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

| Contents | CAT. N° | Size |
|---|---|---|
| * TaqMan Probe Fast qPCR Master Mix | FS-T-1072F | *100 RX |
| ROX Dye (1x) | | 1 vial |

*1 ml = 100 Reactions

## PCR Machines requiring ROX dye

- **High Rox Dye:**
  ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
  - Amount per 50 ul reaction: 1.0 ul (0.6-1.0 ul)
  - Final ROX Concentration: 500nM (300-500nM)
- **Low ROX Dye*:**
  ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene Mx3000, Mx3005P and Mx4000 :
  - Amount per 50 ul reaction: 0.1 ul (0.06-0.1 ul)
  - Final ROX Concentration: 50nM (30-50nM)

***Dilute (1x) Rox : 1:10 with H2O to obtain 0.1X Rox**

## PCR Machines requiring no ROX Dye

**BioRad:** iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon
**Qiagen**: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 **Eppendorf**: Mastercycle realplex - **Illumina**: Eco RealTime PCR System Cepheid: SmartCyler- **Roche**:LightCycler 480,LightCycler 2.0

## 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

## 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.

**Note**

Do not contaminate the TaqMan Probe Fast qPCR Master Mix mix with primers, probe and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

## Recommended 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, probe and water need to be added to the TaqMan Probe Fast qPCR Master 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

| | 20ul reaction | Final conc. |
|---|---|---|
| TaqMan Probe Fast qPCR Master Mix | 10.0ul | 1X |
| ROX Dye (1X) *(optional) | 0.4ul (0.04ul) | 1X (0.1X) |
| 10um Forward Primer | 0.2~2.0ul | 0.1~1.0uM |
| 10 um Reverse Primer | 0.2~2.0ul | 0.1~1.0uM |
| Fluorescence Probe | Variable | ≤500ng/reaction |
| Template** | Variable | NA |
| Water RNase Free | Up to 20ul | |

***Please note "Use of the ROX Reference Dye"**

** Recommended amount of template per PCR Reaction:
- < 50 ng plasmid or,
- < 500 ~ 1,000ng genomic DNA or,
- 2ul of a 100ul single plaque eluate or, one single bacterial colony or,
- 100 ng ~ 1 pg of cDNA

NOTE: In general, use greater than 0.5 uM primers for sensitivity and less than 0.5 uM for specificity.

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
5. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

### PCR Conditions

| Step | Tem p (°C) | Time | Cycle |
|---|---|---|---|
| Initial Denaturation | 95 | *20 sec.~5min. | 1 |
| Denature | 95 | 1 ~ 10 sec. | 35 ~ 40 |
| * Anneal | 55~65 | 20 ~ 50 sec. | |

ATTENTION :ONLY *20 sec ~2min for cDNA,
5 min for genomic DNA

NOTE: Cycling conditions may need to be optimized, epending on different primer and template conditions. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

## Quality Control Analysis

Sensitivity and reproducibility in real-time PCR are tested in parallel reactions containing 10-fold dilutions of nucleic acid template