2xTaqMan qPCR Master Mix（No ROX）

Cat. No.	Product Description	Size
QPT01-00	2xTaqMan qPCR Master Mix (No ROX)	1 ml
QPT01-01	2xTaqMan qPCR Master Mix (No ROX)	5x1 ml
QPT01-02	2xTaqMan qPCR Master Mix (No ROX)	50 ml

Description The 2×TaqMan qPCR master Mix is specially formulated mixture for probe real time PCR (TaqMan, Molecular Beacon etc.). It contains the Hot Start DNA Polymerase, PCR Buffer, MgCl2, dNTPs, or ROX and the stabilizing agent. The Hot Start Taq DNA polymerase in the mix is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers and providing higher specificity of DNA amplification. The activity of the enzyme is turned on when incubation at 95°C in 10 minutes. The unique reaction buffer with the special Hotstar enzyme increases the amplification efficiency, which makes the stronger fluorescent signals, which in turn increase the sensitivity of detecting even single copy sequences.

Storage: -20ºC

Protocol

1. Mixing 2×TaqMan qPCR master Mix by upside down gently a couple of times, avoid foam formation, and spin briefly in a Microcentrifuge.

2. Set up the reaction on the ice as below:

Reaction Mixture	Volume	Final Concentration
2×TaqMan qPCR master Mix	25μl	1×
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
Probe (10 μM)	1 μl	
Template	2 μl	
RNase-Free water	Up to 50μl	

3. Mix the reaction, start PCR

95ºC 10 min
95ºC 15 sec } 30-45 cycles
60ºC 60 sec

Optimization Notes

1. **Primer concentration**: generally at 0.2 μM can get a good result, the user can try from 0.1μ-1.0μM.
2. **Probe concentration**: should be adjusted according to the actuality. User can refer to the instruction of instrument and the probe.
3. **Template:** 10-100ng genomic DNA or 1-10ng cDNA. Since difference template has difference copy number, user should make a gradient dilution to confirm the optimum concentration.

Usage Notes

1. Avoid repeated freezing and thawing, frequently use can be stored at 2-8ºC. For long term storage, store at -20ºC.
2. The functional activity of the enzyme should to be activated in 10 minute incubation at 95°C
3. We recommend to use the two step PCR. If cannot get good result which is caused by the low Tm primer and other reasons, user can use the three step PCR. The annealing temperature we recommend to use is 56-60ºC.