

2xTaqMan qPCR Master Mix (With ROX)

Cat. No.	Product Description	Size
QPT02-00	2xTaqMan qPCR Master Mix (with ROX)	1 ml
QPT02-01	2xTaqMan qPCR Master Mix (with ROX)	5x1 ml
QPT02-02	2xTaqMan qPCR Master Mix (with ROX)	50 ml

Description The 2xTaqMan 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, 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. The ROX in the mix can be used for the correction of the fluorescent signal in the qPCR, will not interfere the reaction in the machine doesn't require ROX as internal control.

Storage: -20°C

Protocol

1. Mixing 2xTaqMan 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
2xTaqMan 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

95°C 10 min	} 30-45 cycles
95°C 15 sec	
60°C 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. The Master Mix containing the ROX needs to avoid the hard light when use and storage.
2. Avoid repeated freezing and thawing, frequently use can be stored at 2-8°C. For long term storage, store at -20°C.
3. The functional activity of the enzyme should to be activated in 10 minute incubation at 95°C
4. We recommend to use the two step PCR. If cannot get good result which is caused by the low T_m primer and other reasons, user can use the three step PCR. The annealing temperature we recommend to use is 56-60°C.



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