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2×Taq Man qPCR MasterMix

Product Number: PCM39

Shipping and Storage

-20°C; If used frequently, store at 2-8°C, avoiding repeated freezing and thawing.

Components

Component	PCM39 5ml	PCM40 5ml	PCM41 5ml
2×Taq Man qPCR MasterMix	5×1ml	5×1ml	5×1mL
50×Low ROX	-	200µl	-
50×High ROX	-	-	200µl
ddH_2O	5×1ml	5×1ml	5×1ml

Description

The 2×Taq Man qPCR MasterMix is a premixed system for qPCR based on probes (TaqMan, Molecular Beacon etc.), and the concentration is 2×. It contains GoldenStar Taq DNA Polymerase, PCR Buffer, dNTPs, and Mg^{2+}. The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription, such as gene expression analysis, copy number analysis, SNP genotyping, etc., and is applicable to the qPCR using different types of probes.

The Golden Star Taq DNA Polymerase in the mixture is a chemically-modified, new efficient hotstart enzyme that does not have polymerase activity at room temperature which prevents nonspecific amplification efficiently, and it is activated by incubation at 95°C for 10 minutes. The combination of a unique PCR buffer system and a hot-start enzyme significantly increases the amplification efficiency of PCR. The fluorescence signal is stronger, and it is more sensitive, which can even detect single copy template. This product can be used to get a wider linear range and more accurate quantification of the target gene. This product is suitable for fluorescent qPCR instruments that do not require ROX as a calibration dye.

Notes

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may comprise product performance. This product can be stored at -20°C in dark for long-term storage. If used frequently in a short time, it can be stored at 2-8°C.

Protocol

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50µl	Final Conc.
2×Taq Man qPCR MasterMix	25µl	1×
Forward Primer，10µM	1µl	0.2µM[1)]
Reverse Primer，10µM	1µl	0.2µM[1)]
Probe，10 µM	1µl	0.2µM[2)]
DNA template	2µl[3)]	
50×Low ROX or High ROX(optional)[4)]	1µl	1×
ddH_2O	up to 50µl	

Note: 1) Usually 0.2μM of primer concentration gives better results, and the final concentration of primers should be between 0.1and1.0μM.

2) The concentration of the probe to be used depends on the type of qPCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or adjust the concentration according the specific application requirements of each probe.

3) Usually the amount of DNA template is 10-100ng for genomic DNA or 1-10ng for cDNA. Template can be gradient diluted to optimize.

4) The excitation optics of the different instruments are different. Add the 50×Low ROX or 50×High ROX according to the instrument.

2. PCR reaction program:

Note! The pre-denaturation reaction of this product must be completed at 95℃ for 10 minutes!

Two-step PCR:

Step	Temperature	Time	
Pre-denaturation	95℃	10 min[1)]	
Denaturation	95℃	15s	35-40cycles
Annealing/Extension[2)]	60℃	1min	

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95℃ for 10 minutes to activate the enzyme.

2) It is recommended to use two-step PCR. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program, and set the annealing temperature between 56-64℃.