Website: www.cwbiosciences.com
Technical Support: service@cwbiosciences.com
Customer Service: 617-800-6495

GoldStar Probe Mixture

Catalog Number: CW0932S CW2625S CW2626S (1 ml)
CW0932M CW2625M CW2626M (5 ml)

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

Kit Components:

Component	CW0932S (1 ml)	CW2625S (1 ml)	CW2626S (1 ml)
2xGoldStar Probe Mixture	1 ml	1 ml	1 ml
50x Low ROX	-	40 µl	-
50x High ROX	-	-	40 µl
ddH_2O	1 ml	1 ml	1 ml

Product Introduction:

The GoldStar Probe Mixture is a premixed system for qPCR based on probes (TaqMan, Molecular Beacon *etc.*), and the concentration is 2×. It contains GoldStar 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 GoldStar Taq DNA Polymerase in the mixture is a chemically-modified, new efficient hot-start enzyme that does not have polymerase activity at room temperature which prevents non-specific 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.

Precautions:

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	50 µl	Final Conc.
2xGoldStar Probe Mixture	25 µl	1x
Forward Primer, 10 µM	1 µl	0.2 µM [1)]
Reverse Primer, 10 µM	1 µl	0.2 µM[1)]
Probe, 10 µM	1 µl	0.2 µM[2)]
DNA template	2 µl [3)]	
Super Pfx DNA Polymerase [4)]	0.5 µl	1 U/50 µl
ddH_2O	Up to 50 µl	

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

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 10-100 ng for genomic DNA or 1-10 ng 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°C for 10 minutes!

Two-step PCR:

Procedure	Temperature	Time	
Pre-denaturation	95°C	10 min [1)]	
Denaturation	95°C	15 sec	} 35-40 cycles
Annealing/Extension [2)]	60°C	1 min	

Note: 1) The hot-start enzyme used in this product must be pre-denatured at 95°C 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°C.