AmpliPLUS Q Probe qPCR Mix (2X)

Cat. No.: BIO-5230-1ml
BIO-5230-5ml

Applications: Amplification and quantification of DNA from a wide range of species. Sample types include genomic DNA, plasmid DNA, cDNA, λDNA, etc.

Quality Control:

Storage Condition: -20°C

1.0 DESCRIPTION

AmpliPLUS Q Probe qPCR Mix (2X) is a 2X master mix for real-time qPCR reactions, utilizing probes such as TaqMan, Molecular Beacon, etc. Consisting of a dual-antibody modified hot-start DNA polymerase in a unique qPCR buffer system, AmpliPLUS Q Probe qPCR Mix (2X) minimizes the amplification of non-specific products and improves single and multiple amplification efficiency through high specificity and sensitivity, with template concentration as low as 30 pg.

AmpliPLUS Q Probe qPCR Mix (2X) displays excellent amplification performance for rich AT and GC templates. The product is stable even after 30 rounds of repeated freeze-thaw cycles and can be stored at 37°C for 14 days.

2.0 PROTOCOL

Points to consider:

1. It is recommended to aliquot the product into multiple tubes for storage, to minimize multiple freeze-thaw cycles.
2. Thaw AmpliPLUS Q Probe qPCR Mix (2X) at room temperature. Keep the tube on ice after thawing. Vortex briefly and spin down contents quickly.
3. Prepare the following reaction mix in a sterile, nuclease-free PCR tube on ice.
4. Due to the aerosol nature of the product, it is recommended to perform setup in a DNA-free environment with dedicated pipette and aerosol resistant tips.
5. Design the probe first before the primer.

General guidelines for Primer Design:

1. Optimal primer length is 20 bases. Do not overlap primer and probe sequence.
2. Maintain GC content between 20 – 80 %.
3. Avoid designing primers with repeated nucleotide sequence. If it is necessary, the number of consecutive G bases must be less than 4.
4. Ensure that there are at most two G and/or C bases in the final 5 nucleotides of the 3’ end.
5. Tm should be maintained at 56°C - 64°C.

Please refer to the table below for a recommended qPCR reaction setup and cycling conditions. Depending on the nature of template, primer design and fragment sizes, further optimization may be necessary to achieve desirable results.

Template DNA recommendations in a 20 µl reaction volume	
Genomic DNA	1 – 100 ng
cDNA	1 – 10 ng

qPCR Reaction Setup			
Components	**25 µl setup**	**50 µl setup**	**Final concentration**
AmpliPLUS Q Probe qPCR Mix (2X)	12.5 µl	25 µl	1X
Forward Primer, 10 µM	0.5 µl	1 µl	0.2 µM (1)
Reverse Primer, 10 µM	0.5 µl	1 µl	0.2 µM (1)
Probe	0.25 µl	0.5 µl	0.1 µM (2)
Template DNA	Variable	Variable	< 1 µg (total DNA)
ddH_2O	Top up to 25 µl	Top up to 50 µl	

(1) If required, primer concentration can be optimized to between 0.1 – 1.0 µM.

(2) Probe concentration is dependent on the instrument, probe type and type of fluorescent label material used. Please refer to instrument manual or probe manual for concentration adjustments.

Recommended qPCR Cycling Condition				
No.	**Steps**	**Temperature**	**Time**	**Cycle**
1.	Initial Denaturation	95°C	5 - 60 s (3)	1
2.	Denaturation	95°C	5 – 15 s	40 - 45
3.	Annealing/Extension	60°C	30 s (4)	

(3) For fast cycling, 5 secs initial denaturation can be deployed. For complex templates, initial denaturation can be extended to 3 min.

(4) It is recommended to use 2-step qPCR cycling conditions. If 3-step qPCR cycling conditions are required due to primers with low Tm values, please set the annealing temperature between 56°C - 64°C.