AmpliPLUS Q SYBR qPCR Mix (2X)

Cat. No.: BIO-5220-1ml
BIO-5220-5ml

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, away from light

1.0 DESCRIPTION

AmpliPLUS Q SYBR qPCR Mix (2X) is a dye based (SYBR Green I) 2X master mix for real-time qPCR reactions. Consisting of a hot-start DNA polymerase in a unique qPCR buffer system, AmpliPLUS Q SYBR qPCR Mix (2X) minimizes the amplification of non-specific products and improves the amplification efficiency through high specificity and sensitivity. AmpliPLUS Q SYBR qPCR Mix (2X) also addresses rich AT and GC templates.

Most qPCR instruments are compatible with the ROX Reference Dye contained in the product and do not require ROX calibration for various systems. AmpliPLUS Q SYBR qPCR Mix (2X) does not create bubbles during vortexing. The product is stable even after 20 rounds of repeated freeze-thaw cycles and can be stored at 37°C for 7 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 SYBR 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. Minimize strong light exposure to the product and setup.

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

For a 20µl reaction volume:		
Components	**Volume (µl)**	**Final concentration**
AmpliPLUS Q SYBR qPCR Mix (2X)	10	1X
Forward Primer, 10 µM	0.4	0.2 µM
Reverse Primer, 10 µM	0.4	0.2 µM
Template DNA	Variable	
ddH_2O	Top up to 20 µl	

Recommended qPCR Cycling Condition				
No.	**Steps**	**Temperature**	**Time**	**Cycle**
1.	Initial Denaturation	95°C	30 s	1
2.	Denaturation	95°C	5 – 15 s	40 - 45
3.	Annealing/Extension	60°C	10 – 30 s *	
		95°C	15 s	
4	Dissolution curve	60°C	1 min *	
		95°C	1s	

- *Set signal acquisition
- Kindly configure the qPCR instrument according to the suggested software settings for melting curve analysis. The above cycling condition was run on QuantStudio™ 5.
- For faster cycling, a 10-sec annealing/extension step can be deployed for amplicons less than 200 bp.

Frequently Asked Questions (FAQ) / Troubleshooting Guide		
1.	• No amplification • Abnormal amplification curve	Sample Issue I. Please check sample purity. Sample must be purified and free of reverse transcriptase residue prior to experiment. II. Re-design primers if they do not match. Instrument Issue I. Depending on the instrument, review the detection settings. II. Review data acquisition time and well location.
2.	• Poor quantification reproducibility	I. Increase template concentration by: i. Increase sample volume loaded. ii. Reduce sample dilution factor. II. Reduce pipetting errors by: I. Frequent calibration of pipettes. II. Avoid small volume pipetting. III. Ensure homogeneity after preparation.
3.	• No Template Control (NTC) amplification	I. If the melting curve (Tm) does not overlap with target gene, it is due to the presence of primer dimers. Re-design primers to better match. II. If the melting curve (Tm) overlaps with target gene, the mix has been contaminated with sample.