Product information

Product Name: 2X qPCR TaqProbe Master Mix

Catalog No: VN710TM and VN711TM

Packing Size: 500 x 25 µl rxns and 250 x 25 µl rxns

Shipping Condition: Ice pack

Storage Condition: -20^{0}C

Product Description:

2X qPCR TaqProbe Master Mix is specifically designed for real-time quantitative analysis of DNA target genes. The components of the mix have been optimized for high sensitivity, specificity, and low background. The hot-start and inhibitor-resistant Taq polymerases included in the mix significantly reduce non-specific PCR amplification and false negatives due to residue of PCR inhibitors, which can be commonly observed with other commercial Taq polymerases.

It is important to note that some PCR instruments require the use of a passive reference dye (ROX). This Master Mix does not contain a Passive Reference Dye ROX, but you may add ROX to the reactions if your instrument requires it. For more information on how to use ROX with this Master Mix, please refer to the table provided.

Final concentration of ROX dye in PCR	Recommended Cyclers
500 nM	ABI® PRISM 7000,7300,7700,7900HT, 7900Fast, StepOnePlus™, StepOne™
50 nM	ABI® PRISM 7500, 7500Fast -Stratagene® Mx3000, Mx3005P, Mx4000
No	BioRad® iCycler®, iQ ™5, MyiQ™ BioRad® CFX96 Roche LightCycler® 480 MJ Research Opticon™ And Opticon™ 2, MJ Research Chromo® 4 Corbett Rotor-gene® 6000, 3000 DNA Engine Option® 2 and Chromo 4™ Eppendorf® Realplex

VitaNavi Technology LLC. 613 Big Bend Rd, Unit 941, Ballwin MO 63021. Toll free (US and Canada): (800) 586-6927; Phone: (314) 499-8280. Fax: (866) 302-5897. Email: support@vitanavitech.com. Website: https://www.vitanavitech.com.

Protocol:

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
2X qPCR TaqProbe Master Mix	12.5 µl	
Left Primer	Variable	0.2-0.4 µM
Right Primer	Variable	0.2-0.4 µM
Probe	Variable	0.2-0.4 µM
ROX (not included)	Variable (refer to the table above)	0-500 nM
DNA template†	Variable	0.1-100 ng
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

†DNA amount depends mostly on genome size and target gene copy number.

Typical Cycling Parameters

Three steps

Initial denaturation	95^0C	2-5 min
Then followed by 35-45 cycles		
Denaturation	94^0C	20-30 sec
Annealing	50^0C to 68^0C	20-30 sec
Plate read	Follow instrument guideline	
Extension	70^0C	0.5-2 min

Two steps

Initial denaturation	95^0C	2-5 min
Then followed by 40-45 cycles		
Denaturation	94^0C	20-30 sec
Annealing / Extension	60^0C to 65^0C	0.5-2 min
Plate read	Follow instrument guideline	

Troubleshooting guide

No signal	Please check your PCR settings to ensure that you have not missed any necessary components. You may want to try a gradient annealing temperature to identify the optimal temperature for your target. Additionally, it appears that the annealing and extension times may be too short. Please refer to the protocol for the recommended times. If you are still experiencing issues, it is possible that the primers or RNA template are degraded or that the quantity of template is too low.
Low signal	If you are experiencing a low signal in your real-time PCR, there are several potential solutions. First, you may want to consider increasing the number of PCR cycles. Additionally, it is important to ensure that the reagents are stored properly to maintain their stability. It is possible that the primers are not optimal, in which case you may need to redesign them. Finally, the length of the PCR product may be contributing to the low signal; optimal PCR products are typically between 100-200 bp in length.
Unexpected variation	If you are experiencing unexpected variations in your real-time PCR results, there are a few steps you can take to address the issue. First, check your pipette to ensure that it is calibrated and accurate. Additionally, you should verify whether you need to add a ROX reference dye that is suitable for your specific PCR cycler model (please refer to the manufacturer's instructions or the provided reference table).

Note: This product is for R&D use only

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