Differentiating Research

Product Details

VEGA for REAL TIME (2X)

Product Overview

VEGA for Real Time (2X) is a convenient, pre-mixed solution designed for reverse transcription quantitative PCR (RT-qPCR) of RNA templates using probe-based detection chemistries like TaqMan® 5'-hydrolysis probes. This ready-to-use master mix streamlines the process by combining first-strand cDNA synthesis and PCR amplification within the same tube, eliminating the need for intermediate steps. Ideally suited for sensitive quantification of RNA viruses or low-abundance RNA targets in both single and multiplexed RT-qPCR applications, as well as high-throughput gene expression studies.

Catalog Details

R6323 20µL X 200 Reactions
R6324 20µL X 1000 Reactions

Storage

- Store the mastermix at - 20 ℃ when arrived

ROX Compatibility

- High ROX Instruments - Use the vial **ROX Reference Dye (H)** for instruments like Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, StepOne, StepOnePlus and other similar instruments which require high ROX
- LOW ROX Instruments - Use the vial **ROX Reference Dye (L)** for instruments like Applied Biosystems 7500, 7500 Fast Real time systems, Stratagene, QuantStudio Systems and other similar instruments which require low ROX
- NO ROX Instruments - Qiagen Rotor Gene, Roche LifeCycler, Biorad CFX96, CFX 384, Eppendorf MasterCycler and other similar instruments would not require ROX.

RT-PCR Protocol

ADD ALL THE BELOW COMPONENTS TO A SINGLE TUBE

Components	Example for 20µL reaction	Final Concentration
Template RNA	Variable	<100ng of Total RNA
Forward Primer (10µM)	0.8 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	0.1 - 1µM
ROX Reference Dye (Refer instruments)	0.4 µL	High - 500nM; Low - 50nM ; No ROX
PROBEs	Variable	0.1-0.5µM
VEGA for Real Time Mastermix (2X)	10 µL	1X
Nuclease Free Water	Upto 20 µL	

PCR Program

Step	Temperature	Time	Cycle
Reverse Transcription	42 ℃★	10 minutes	1
Initial denaturation	95 ℃	2 minutes	1
Denaturation	95 ℃	5-10s	35 to 45
Annealing */Extension	60 ℃ to 65℃	20 - 30s	

★ *RT temperature can be increased upto 60℃ based on complex RNA structure.*

Critical Note

- cDNA quality depends on the initial RNA template used. Few desired genes might have very low or very high transcripts based on the cell's growth conditions. Users can empirically choose 25 to 45 cycles in the PCR step to obtain desired amplification. For very low copy transcripts use 45 cycles and for a high copy transcripts you can use 30 cycles.
- Reverse Transcription temperature can be increased upto 60℃ to reduce non-specific amplification.
- For Ver low copy transcripts, Reverse transcription time can be increased upto 15 minutes.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 98% purity for all the enzymes used.
2. **Performance testing:** In a 20µL reaction, 10µL of mastermix was used to amplify 10 ng of total RNA template from Bitter-gourd Plant with appropriate primers. PCR was run with 30 cycles resulted in a single product (~400bp) confirmed by melt curve analysis and also same sample was re-confirmed on 1% agarose gel electrophoresis with EtBr.
3. **Nuclease tests:** No contamination of endo or exonucleases were detected.

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Other Products

Leo Prime Mastermix > > For end point PCR/Colony PCR/genotyping
Polaris -AMP (2X) >> For TaqMan probe based qPCR application/Multiplexing
VEGA SYBR Mastermix >> One Step mastermix containing SYBR Green I
DNA Ladders >> For gel electrophoresis
Taq DNA Polymerase
Reverse Transcriptase
Ribonuclease inhibitors
RNA Builder products