

#### **Product Overview**

VEGA SYBR® mastermix eliminates the need for separate reactions, saving time and reducing experimental variability. By integrating cDNA synthesis and qPCR amplification, researchers can achieve sensitive and accurate gene expression analysis with ease. VEGA Sybr Green Mastermix offers convenience, efficiency, and reliability, empowering researchers to focus on their scientific discoveries rather than laborious experimental setups.

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Storage

- R6325
   20μL X 200 Reactions

   R6326
   20μL X 1000 Reactions
- Store the mastermix at 20 ℃ when arrived

# **ROX** Compatibility

- <u>High ROX Instruments</u> 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
- <u>LOW ROX Instruments</u> 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
- <u>NO ROX Instruments</u> 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
VEGA ™ SYBR Mastermix (2X)	10 µL	1X
Nuclease Free Water	Upto 20 µL	

# PCR Program

Step	Temperature	Time	Cycle
Reverse Transcription	42 °C	10 minutes	1
Initial denaturation	95 <b>℃</b>	2 minutes	1
Denaturation	95 <b>°C</b>	5-10s	35 to 45
Annealing */Extension	55 ℃ to 65℃	20 -30s	
Melt Analysis	65℃ to 95℃	variable	

• GREEN CHANNEL TO BE SELECTED IN REAL TIME PCR FOR DETECTION.



#### **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 30 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°C to reduce non-specific amplification.

## **Quality Control Assays**

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in  $\ge$  95% purity for all the enzymes used.

2. **Performance testing:** In a 20µL reaction, 10µL of mastermix was used to amplify 10ng of total RNA template from human HEK cell-lines with appropriate primers. PCR was run with 35 cycles resulted in a single product (~150bp) confirmed by melt curve analysis and also same sample was re-confirmed on 1% agarose gel electrophoresis with SafeStain Green.

3. Nuclease tests: No contamination of endo or exonucleases were detected.

#### Any Technical Help?

Please write to us at <u>info@dxbidt.com</u>. Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

## **Order Related Queries**

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## **DX/DT PRODUCTS**

VEGA - For Gel Based assay VEGA - For Probe based assays VEGA- PCR KIT with control RNA Polaris - AMP -- For TaqMan based assays (DNA) Polaris - For SYBR based assays(DNA) DNA Ladders Premium enzymes