

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 ℃ | 2 minutes | 1 |
| Denaturation | 95 °C | 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.

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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