Differentiating Research

Product Details

VEGA™ OneStep With control RNA(2X)

Product Overview

VEGA ™ One Step RT-PCR mix is a pre-formulated mixture of reagents used for reverse transcription polymerase chain reaction (RT-PCR) in a single step. The mix (2X) contains dNTPs, stability reagents and reaction buffer. It simplifies the RT-PCR process by eliminating the need for separate reverse transcription and PCR steps. The enzyme mix provided contains Reverse Transcriptase, Ribonuclease inhibitor and Taq DNA Polymerase(HotStart). The master-mix can be used for the detection and quantification of RNA targets, including viral RNA, in a variety of sample types. It is highly sensitive and specific, and can detect low levels of RNA with high accuracy. VEGA ™ One Step RT-PCR mix is widely used in molecular biology research and diagnostic applications.

This kit includes all reagents necessary for reverse transcription of RNA to cDNA and cDNA amplification using PCR. The kit also comes with a control RNA (total RNA from a plant) with appropriate forward and reverse primers. So you can set up a control reaction with ease. The final amplicon is about 400bp length.This product is optimized for gel electrophoresis based end point PCR quality checks only.

Kit Components

- VEGA OneStep Mastermix (2X) , 2 mL (20 µL X 200 Reactions)
- Enzyme mix , 200µL
- Nuclease Free Water, 2 mL
- Control RNA, Extra pure (100µL)
- Forward Primer, 10µM (100µL)
- Forward Primer, 10µM (100µL)

Catalog Details

R6327	2 mL (20µL X 200 Reactions)
R6328	5 mL (20µL X 500 Reactions)

Storage

At - 20 ℃

OneStep Protocol

Components	Example for 20µL reaction	Final Concentration
Template RNA	Variable	<1µg
Forward Primer (10µM)	0.8 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	0.1 - 1µM
VEGA ™ for End Point One Step (2X)	10 µL	1X
Enzyme Mix	1µL	1 to 2µL
Nuclease Free Water	Upto 20 µL	

OneStep Protocol for CONTROL RNA

Components	Example for 20µL reaction	Final Concentration
Control RNA	1 µL	~25ng
Forward Primer (10µM)	0.8 µL	0.1 - 1µM
Reverse Primer (10µM)	0.8 µL	0.1 - 1µM
VEGA ™ One Step (2X)	10 µL	1X
Enzyme Mix	1µL	1 to 2µL
Nuclease Free Water	Upto 20 µL	

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PCR Program (For shorter fragments ≤ 1KB) 2-Step PCR = **PCR Program for Control RNA**

Step	Temperature	Time	Cycle
Reverse Transcription	42 ℃	15 minutes	1
Initial denaturation	95 ℃	2 minutes	1
Denaturation	95 ℃	5-10s	30 to 45
Annealing */Extension	55 ℃ to 65℃	20 - 30s	
Final Hold	4℃	variable	

PCR Amplicon Length for control RNA

400bp

PCR Program (For Long fragments ≥ 1KB) 3-Step PCR

Step	Temperature	Time	Cycle
Reverse Transcription	42 ℃	15 minutes	1
Initial denaturation	95 ℃	2 minutes	1
Denaturation	95 ℃	5-10s	30 to 45
Annealing	55 ℃ to 65℃	20 - 30s	
Extension	72℃	1 min/kb	
Final Extension	72℃	10 minutes	

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 40 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.
- This One Step Mastermix is optimized for ***End Point PCR*** reactions where no flourscence dye is used.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity for all the enzymes used.
2. **Performance testing:** In a 20µL reaction, 10µL of mastermix was used to amplify 25ng of RNA template (from a plant) with appropriate primers (given in the kit). PCR was run with 30 cycles resulted in a single product (400bp) confirmed by on 1% agarose gel electrophoresis with SafeStain Green OR EtBr.
3. **Nuclease tests:** No contamination of endo or exonucleases were detected.

Other products

R6323	VEGA ™	One Step for Real Time Probe PCR (2X)
R6325	VEGA ™	One Step SYBR+ Mastermix (Real Time qPCR)
R6329	VEGA ™	One Step 4X for Real Time PCR (4X)

Any Technical Help ?

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