

VEGATM for End Point PCR (2X)

Product Overview

VEGATM 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. VEGATM One Step RT-PCR mix is widely used in molecular biology research and diagnostic applications.

Catalog Details

R6321 2 mL (20 μ L X 200 Reactions)
R6322 10 mL (20 μ L X 1000 Reactions)

Storage

- Store the mastermix at - 20 °C when arrived

RT-PCR Protocol

ADD ALL THE BELOW COMPONENTS TO A SINGLE TUBE

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

PCR Program (For shorter fragments \leq 1KB) 2-Step PCR

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

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

Step	Temperature	Time	Cycle
Reverse Transcription	42 °C	15 minutes	1
Initial denaturation	95 °C	2 minutes	1
Denaturation	95 °C	5-10s	30 to 45
Annealing	55 °C to 65°C	20 - 30s	
Extension	72°C	1 min/kb	
Final Extension	72°C	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°C to reduce non-specific amplification.
- This One Step Mastermix is optimized for End Point PCR reactions where no fluorescence dye is used.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in $\geq 95\%$ purity for all the enzymes used.
2. **Performance testing:** In a 20 μ L reaction, 10 μ L of mastermix was used to amplify 50ng of RNA template (from Human HEK cell lines) with appropriate primers. PCR was run with 30 cycles resulted in a single product (650bp) 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.

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.

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