v i v a n t i s

DNA AMPLIFICATION PRODUCT

2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix

Taq RTMM

Lot	:
Expiry Date	:
Supplied with	:	1ml **2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix ***
		2ml of Nuclease-free Water

Store at -20°C

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Description :

2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix offers rapid and sensitive end-point detection of RNA templates in a single step. 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix is an optimized ready-to-use 2X concentrated RNA amplification mixture containing M-MuLV Reverse Transcriptase, RNase Inhibitors, *Taq* DNA Polymerase, reaction buffer and dNTPs. It contains all the components required for routine RNA amplification except template and primers. Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase has the absence of RNase H activities that enhance the synthesis of long cDNAs and amplification of long transcripts. 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix allows one-step RT-PCR using only gene-specific primers. 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix contains the inert red dye and stabilizers that allow the direct loading of final PCR products onto gels for electrophoresis. The red color dye migrates at approximately 400bp on 1% agarose gel in 1X TBE Buffer.

Features :

- Suitable for all routine RNA amplification applications.
- Reduces set-up time and buffer-dye mixing
- Minimizes potential contamination due to reduced number of tests and pipetting steps.
- Easy confirmation of complete mixing
- No additional loading dye needed
- direct loading of final products onto gels.

Storage and Stability :

- 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix is stable at -20°C for one year or 4°C for 6 months if properly stored.
- 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix is stable for 20 freeze-thaw cycles. To avoid frequent freeze-thaw, keeping small aliquot at -20°C is recommended.
- For daily use, keeping an aliquot at 4°C is recommended.

Quality Control :

All preparations are assayed for contaminating endonuclease, exonuclease, and non-specific RNase activities. Functionally tested in DNA amplification.

Recommended Protocol for 2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix:

Gently mix all solutions after thawing. Spin down briefly and keep on ice.
Add the following components in a 0.2ml thin walled PCR tube on ice.

For 20µl reaction volume:

Reagent:	Volume	Final Concentration
2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix	10µl	*1X
Water, nuclease free	Adjust final volume to 10µl	
RNA template	Variable	0.02 – 5µg
Primers (Fwd / Rev)	Variable	0.1 – 1µM each

Incubate primer-RNA template mix at 65 °C for 5 min before adding
2X ViRed OneStep *Taq* ReverseTrans PCR Master Mix.

* 40U M-MuLV Reverse Transcriptase, RNase Inhibitors, 1.5U *Taq* DNA Polymerase, 1X PCR Buffer, 0.2mM dNTPs Mix, inert red dye and enhancers.
**Higher reaction volume may be achieved provided that the same final concentration of each reaction component is maintained. The number of tests will be reduced if the reaction volume more than 20µl.

Cycling Conditions (100bp - 500bp)

cDNA Synthesis	42°C for 10 – 30 minutes	
Initial Denaturation	94°C for 2 minutes	
Denaturation	94°C for 15 seconds	
Annealing	50 -68°C for 1 minute	25 -40 cycles
Final Extension	72°C for 5 minutes	

Cycling Conditions (300bp - 5kb)

cDNA Synthesis	42°C for 10 – 30 minutes	
Initial Denaturation	94°C for 2 - 5 minutes	
Denaturation	94°C for 30 seconds	
Annealing	50 -68°C for 30 seconds to 1 minute	25 - 40 cycles
Extension	72°C for 30 seconds to 2 minutes	
Final Extension	72°C for 5 minutes	

*This protocol may change depending on the template RNA and primers used.