Technical Bulletin 1/1.

Product Information:

One step RT-PCR Master Mix (2x) (Cat.: R2120) storage: -20°C

Size: 1.25 mL (100 Reactions)

Description:

The ready-to-use one step RT-PCR Master Mix contains 2x M-MLV Reverse Transcriptase, Taq DNA Polymerase, recombinant RNase Inhibitor, Ultrapure nucleotides, magnesium and PCR reaction buffers, designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA. Simply mix the RNA template, primers, and RNase free water with the RT-PCR master mix and the reactions are ready to cycles.

Procedure:

Prepare a single reaction (total volume: 25uL) in a 0.2 or 0.5 mL microtube.

Component Volume (µL) Final Concentration
One step RT-PCR Master Mix (2x) 12.5 1x
RNA Template 0.1-1 determined by user
Forward primer (5µM) 1 200nM
Reverse primer (5µM) 1 200nM
PCR grade/RNaes-free water up to 25 μL

Setup typical thermal cycling parameters

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1. Reverse Transcription (RT) step: 45°C 15 minutes					
2. Enzyme activation step: 95°C 2 minutes					
3. 25-40 cycles:					
Denature 95°C 30 seconds					
Annealing X°C 30 seconds dependent on Tm of primers					
Extension 68°C 1 minute (1min per kb amplicon)					
4. Final extension 68°C 1 minute (1min per kb amplicon)					
5. Hold 4°C					

Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.