

CleanAmp™ One-Step RT-PCR 2X Master Mix

Catalog # L-5104

L-5104-025 (25 reactions)

L-5104-100 (100 reactions)

CleanAmp™ One-Step RT-PCR 2X Master Mix is an optimized, ready-to-use mix of CleanAmp™ dNTPs, M-MLV (RNase H-) reverse transcriptase and *Taq* DNA polymerase in reaction buffer suited for one-step reverse transcription PCR in a single tube. CleanAmp™ One-Step RT-PCR 2X Master Mix has been optimized for simultaneous amplification of multiple targets. Simply add primers, template RNA and water.

QC Analysis

Functional Assay; Pass

Tested in standardized PCR assay for efficiency and specificity.

Handling & Use

Store at -20 °C

Stable to 5 freeze-thaw cycles. Exposure to ambient temperatures during shipping does not adversely affect product performance.

CleanAmp™ Products: Patent Pending | RESEARCH LICENSE AGREEMENT

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Protocols

One-Step RT-PCR (25 µL)

1. Thaw CleanAmp™ One-Step RT-PCR 2X Master Mix and primers and place on ice. Thaw RNA template on ice. Note: Do not vortex CleanAmp™ One-Step RT-PCR 2X Master Mix. Mix thoroughly by pipetting up and down and collect by pulse centrifugation.
2. Prepare a reaction mixture containing all components except for the RNA template. Add CleanAmp™ One-Step RT-PCR 2X Master Mix, primers and sterile de-ionized water as shown in Table 1 into thin-walled PCR tubes. Keep on ice.
3. Mix the reaction mixture gently to protect the enzyme, by pipetting up and down. Do not vortex. Pulse spin if necessary.
4. Add the appropriate volume of template RNA¹ to reach a reaction volume of 25 µL.
5. Pulse spin to remove bubbles and collect reaction solution at bottom of PCR tube.
6. Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions for standard thermal cycling:
 47 °C for 30 min²
 94 °C for 10 min
 [94 °C for 15 sec; 48-60 °C³ for 30 sec; 72 °C for 1-2 min⁴]
 30-40 cycles
 72 °C for 5 min
7. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

Table 1

Component	Final Concentration (25 µL reaction)	Volume per reaction
CleanAmp™ One-Step RT-PCR 2X Master Mix	1X	12.5 µL
Forward Primer	0.5 µM	Variable
Reverse Primer	1.0 µM	Variable
RNA Template	Variable	Variable
Sterile De-ionized Water	Up to 25 µL	Up to 25 µL
Total Volume (µL)	25 µL	25 µL

¹ This Master Mix can be used for both singleplex and multiplex assay formats, where multiplexing up to a fourplex has been demonstrated. Amount of starting material can range from 50 pg-1 µg of total RNA.

² The duration of the RT step can be shortened to as little as 10 minutes. 30 minutes is recommended for initial evaluation. The maximum recommended temperature for the RT step is 48 °C.

³ The annealing temperature should be chosen for optimal PCR performance through primer design software or experimentation.

⁴ Extension times may need be increased/optimized for longer targets.

Real-Time PCR

CleanAmp™ One-Step RT-PCR 2X Master Mix has been successfully adapted for real-time detection using intercalating dye and probe-based detection. Please refer to the instrument manufacturer for specific protocols.

Real-time PCR may be proprietary. No license is conveyed expressly or by implication to the purchaser by purchase of any TriLink BioTechnologies products.