2x One-tube RT-PCR mix (Hot start)

Cat No: ORP01H
Size: 50 rxn / 1.25 ml

Description :
The One-tube RT-PCR Hot Start kit are designed especially for simple and effective . The reverse transcription step is directed by M-MLV RT (H-). The PCR reaction is by a chemically modified “Hot-Start” Taq , is activated by heating up the reaction 10 min at 94°C, the Hot start Taq enzyme is activated. An especially reaction buffer provides both for Reverse Transcriptase and for Hot Start Taq DNA Polymerase.

Storage conditions: long time at -20°C

RNA Isolation:
High-quality intact RNA is essential for successful synthesis of full-length cDNA and yield of long RT-PCR products. Total and poly(A)+ RNA can be rapidly isolated and purified using Bioman Total RNA reagent. Oligo(dT)-selection for poly(A)+ RNA is typically not necessary, although including this step may improve the yield of specific cDNA templates. RNA samples with an OD260/280 of 1.8–2.0 are optimal.
The one-tube RT-PCR system can ready detect RNA targets of 0.1–6 kb in length using 10–200 ng of total RNA or 0.1–10 ng of poly(A)+ RNA.

Protocol: 1. Add the follow reagents

Component	Volume
Primer1(100nmol)	1 ul
Primer2(100nmol)	1 ul
RNA10-500ng	1 ul
2x one tube RT-PCR mix	25 ul
ddH2O	Up to 50 ul
Total	50 ul

2. Vortex the reaction gently without creating bubbles.
3. Place the reaction in a thermal cycler. Run the following thermalcycling program

Step	Temperature °C	Time min	Cycle
RT reaction	37-50	30-120	1
Initial enaturation	94	10	1
Denaturation	94	0.2-1	30-45
Annealing	50-68	0.2-2	
Extension	72	1min/kb	
Final extension	72	1-10	1

Analyzing the RT-PCR Products
Analyze the RT-PCR products by 1.0% (w/v) agarose gel electrophoresis. The products will be ready visible by UV transillumination of the ethidium bromide-stained agarose gel. Store the reaction products at –20°C until needed.
The RT-PCR products may be purified using the Bioman Total RNA reagent Kit.

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