2x One-tube RT-PCR mix

Cat No	Pack size
OR0050	50 rx 1.25 ml
OR0100	100 rx 2x1.25 ml
OR0500	500 rx 10x1.25 ml

Description:

The One-tube RT-PCR mix 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" zymTaq , 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: -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 the Zymeset 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 mix can ready detect RNA targets of 0.1–6 kb in length using 1pg–200 ng of total RNA or 0.1pg–1ng 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
ddH_2O	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 thermal cycling program

Step	Temperature °C	Time min	Cycle
RT reaction	42-50	30-120	1
Initial Denaturation	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

4. Incubate the mixture at 42-50°C during 30-120 min. The time of reaction depends on the length of cDNA, 30 min is for cDNA in range of 500 bp, 120 min is for cDNA more then 1.5 kb. The temperature of the reaction depends on the structural features of RNA. Use increased temperature (up to 50°C) for the highly structured RNA.

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.

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