One Step Quick RT-PCR Kit

 # G7113A 100 reactions

Storage: -20^{0}C

Spin tubes briefly before use

Kit Contents	Amount
Enzyme Mix 1	100µl
Enzyme Mix 2	100µl
10X One-Step Reaction Buffer	200µl
100mM $MgCl_2$	50µl
pdNTP mix, 10 mM	100µl
Nuclease-free H2O	1.5ml

Description

GCC Biotech offers One Step Quick RT-PCR Kit for sensitive and end point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers. It is fast and easy one tube set up. One Step RT-PCR Kit includes dNTPS, one step 10 X reaction buffer, enzyme mix with reverse transcriptase and proof reading hot start DNA polymerase and ribonuclease inhibitor, resulting in high-yield amplification with minimal optimization.It is recommended in detection or quantification of mRNA from, a single step.

Protocol

1. Mix the following components, except RNA, in sterile RNase-free microfuge tubes.

Component	Volume
10 X One-Step Reaction buffer	2 µl
100mM $MgCl_2$	0.5 ul
dNTP Mix, 10mM	1 µl
Enzyme Mix 1	1 µl
Enzyme Mix 2	1 µl
Gene-specific Forward Primer (10 µM)	1 µl
Gene-specific Reverse Primer (10 µM)	1 µl
Nuclease-free H2O	X µl
Total RNA (50ng - 1 µg*)	X µl
Total volume	20 µl

2. Add RNA template last, and start reactions immediately, as follows:

Cycle step	Temp.	Time	Cycles
Initial Denaturation	55°C	5 min	1
Reverse Transcription	55°C	90 min	1
Enzyme 1 inactivation	70°C	15 min	1
Initial Denaturation	94°C	5 min	1
Denaturation **Annealing** **Extension**	94°C Tm 72°C	30 sec 30sec 60sec/1kb	30 cycles
Final Extension	72°C	10 min	1
Hold	4°C	∞	1

Storage Buffer

The enzymes are supplied in: 50mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM EDTA, 1mM DTT, 0.1% (v/v) Nonidet P40, 50% glycerol (v/v).

10X One-Step Reaction buffer

500 mM Tris-HCl, pH 9.2; 500 mM $(NH_4)_2SO_4$; 30 mM $MgCl_2$; 1% Tween 20.

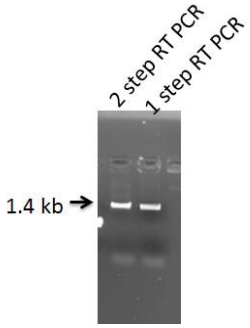

Figure 1. RT PCR product of the *rbc*l gene from *zea mays*. RNA template used 50ng. 10 µl pcr product loaded in1% agarose gel.

Note:

1. Please ensure the quality of your rna (devoid of any contaminating gDNA) to get rid of any false result.
2. To get single desired band, designing of unique primers for the target template is absolutely needed.
3. Requirement of RNA during reaction depends upon the copy number of the template. In case of low abundant RNA, optimization of RNA conc. also crucial.
4. In control experiment 1.4 Kb product (against rbcl rna from plant) will obtain after I step RT PCR reaction.