

Reaction Condition

1. First Strand cDNA synthesis for RT-PCR

① Contents

0.5-5 μ g	total RNA or 50-500 ng poly(A) ⁺ RNA
5 pmoles	oligo(dT) ₁₂₋₁₈ or 5 pmoles gene specific primer or 25 pmoles random primers
4 μ l	5X Buffer
2 μ l	10 mM dNTPs (not provided)
1 μ l	ReverTra Ace (100 units)
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20 μ l	Total Volume

② Incubation

30°C*, 10min.

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42°C, 20-60min.

* If random primers are used, this incubation step is required before the 42°C incubation.

③ Inactivation

99°C, 5min.

2. PCR Amplification

① Contents

~20 μ l*	1st strand reaction	*The volume of 1st strand reaction added should be under 20% of PCR mix.
10 μ l	10X Buffer	**Adjust to 0.2mM as final concentration depending on volume of 1st strand reaction.
0.2 mM**	dNTPs	
10 pmoles	amplification primer1	
10 pmoles	amplification primer2	
5 units	rTaq DNA polymerase or 2.5 units of KOD Dash DNA polymerase	
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100 μ l	Total Volume	

Layer 2~3 drops of silicon oil over the reaction. (if needed)
Perform ~40 cycles of PCR.

② PCR Condition

PCR condition depends on primer and template and must be optimized for each primer template pair.

For G3PDH control reaction, temperature parameters are following.

(1)rTaq DNA Polymerase

94°C 30sec., 60°C 30sec., 72°C 90sec., 35 cycles

(2)KOD Dash DNA Polymerase

94°C 30sec., 60°C 2sec., 72°C 30sec., 35 cycles

G3PDH control primer F 5'-ACCACAGTCCATGCCATCAC-3'

G3PDH control primer R 5'-TCCACCACCCTGTTGCTGTA-3'