

OneStep RT-PCR kit

OneStep RT-PCR is convenient system for setting up one-tube RT-PCR reactions. It contains master enzyme mix including highly processive dART reverse transcriptase, high fidelity OptiTaq DNA polymerase and unique RNase Inhibitor working well at elevated temperature. Master Buffer contains optimized 2 x buffer including dNTPs, stabilizers and reaction enhancemts.

Cat. No.SizekE0803-0125 reactionsfrE0803-02100 reactions

Kit is designed to sufficient amplification DNA from any RNA with high specificity and sensitivity in a one-step process. Our system is dedicated for analytic as well as cloning purposes.

Kit Components:

Reagents are provided for 25 or 100 RT-PCR reactions of 25 µl each.

		<u>Component</u>	<u>25 rxn kit</u>	<u>100 rxn kit</u>
Quality Control:		2 x master buffer mix	350 µl	1.4 ml
All proparations are accave	accound for	Master Enzyme mix	25 µl	100 µl
All preparations are assayed i	u 101	Nuclease-free Water	1.0 ml	4 x 1.0 ml
contaminating endonuclease	aminating endonuclease and			

Protocol:

activities.

1. In 0.2 PCR tube, combine as follows:

Storage Conditions: Store at -20°C

exonuclease and nonspecific RNase and single- and double-stranded DNase

Component:	Amount:
2 x master buffer mix	12.5 µl
sens primer 10 µM	1 µl
reverse primer 10 µM	1 µl
RNA (10 ng-2 µg)	x µl
Master Enzyme mix	1 µl
Nuclease-free Water	to 25 µl

2. Gently mix reaction by pipeting or if needed briefly centrifuge.

3. Transfer the sample to thermal cycler. Incubate as follows:

30 min at 50°C for followed by standard PCR with annealing temperatures suitable for the primers.

5 min	94°C pre-denaturation	
30 s	94°C denaturation	
30 s	50-65°C annealing	30-40 cycles
1 min/1kb	72°C extend	
5 min	72°C final extension	

4. Analyze 5-20 µl of RT-PCR sample by agarose gel electrophoresis with suitable molecular markers.