One-Step RT-PCR

Components	RT01-01	RT01-02
DNA Polymerase (5 U/ul)	50 ul	200 ul
PowerScript™ RTase (200 U/ul)	25 ul	100 ul
2X One-Step RT-PCR Buffer	1 ml	3 x 1 ml
Size	**25 rxns**	**100 rxns**

Product Description

One-Step RT-PCR Kit contains all necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube. Specifically, this One-Step RT-PCR kit contains EasyScriptTM RTase and BestaqTM DNA Polymerase in a convenient format for highly sensitive and specific RT-PCR using any RNA template. Our proprietary RT-PCR buffer contains stabilizers and enhancers that optimize the two reactions in a "single step". Together with a specially formulated RT-PCR buffer, this One-Step RT-PCR kit offers the end-users an efficient, easy to use and reliable alternative to conventional "two-step" sequential RT-PCR.

Storage Conditions

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.

Protocol

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR, and subsequent reaction analysis should be performed in separate areas. The use of clean pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Prepare the following reaction mixture in a PCR tube on ice:

Components	Volume	Concentration
Total RNA or poly(A)$^+$ mRNA	Variable	1 ng – 2 µg 1 pg – 2 ng
2X One-Step RT-PCR Buffer	25 ul	1X
RTase (200 U/ul)	1 ul	200 U/rxn
DNA Polymerase (5 U/ul)	2 ul	10 U/rxn
Forward Primer (10 uM)	2.5 ul	500 nM
Reverse Primer (10 uM)	2.5 ul	500 nM
Nuclease-free H_2O	up to 50 ul	-

Note: *Gene-specific primers should be used.*

2. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
3. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification automatically. The following cycling conditions were established using a DNA Thermal Cycler 2400 (Perkin Elmer) and may have to be altered for other thermal cyclers.

Steps	Temperature	Duration	Cycle(s)
cDNA Synthesis	42°C	30 mins	1
Initial Denaturation	94°C	3 mins	1
Denaturation	94°C	30 secs	30-35
Annealing	55°C	30 secs	
Extension	22°C	1 kb/min	
Final Extension	72°C	5 mins	1
Holding	4°C	-	1

Note:

- The thermal cycling program listed above is optimized for primers with an annealing temperature at 55°C.
- An optional touchdown thermal cycling program can also be used to replace the steps after the initial cDNA synthesis in the table above.

4. Analyze the amplification products by agarose gel electrophoresis and visualize the nucleic acids via ethidium bromide or Safe DNA Gel Stain (Cat. No. SAFE01-01). If 2X One-Step RT-PCR with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.

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