



MegaFi™ Pro One-Step RT-PCR Kit

Cat. No. G597

Store at -20°C.

Product Description

MegaFi™ Pro One-Step RT-PCR Kit is an enzyme mix of OneScript® Hot Reverse Transcriptase and MegaFi™ Pro Fidelity DNA Polymerase with RNaseOFF Ribonuclease Inhibitor, gel loading dye, and all other necessary reagents in a single One-Step 2X RT-PCR Buffer for both **highly sensitive and specific reverse transcription and high fidelity PCR amplification in a single reaction tube** from any RNA template. The kit provides flexibility in choosing desired primers for use with a proprietary RT-PCR buffer containing stabilizers and enhancers that optimize the two reactions in a "single step". This kit offers simple, efficient reaction setup, and is a reliable alternative to conventional "two-step" sequential RT-PCR.

Product Component	Quantity	Part No.
RT-PCR Enzyme Mix	100 rxn (400 µl)	P597-1
2X One-Step RT-PCR Buffer	2 x 1.25 ml	P597-2

Protocol

Reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

Component	Volume
2X One-Step RT-PCR Buffer	25 µl
RT-PCR Enzyme Mix	4 µl
Forward Primer (10 µM)	2.5 µl
Reverse Primer (10 µM)	2.5 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/rxn)
Nuclease-free H ₂ O	up to 50 µl

2. Gently mix the reaction and briefly centrifuge.
3. Thermocycling conditions for standard PCR:

Step	Temperature	Time
cDNA Synthesis	60°C	15 min
Initial Denaturation	95°C	5 min
25 – 35 Cycles	95°C 50-70°C 72°C	15 sec 15 sec 20-30 sec/kb ¹
Final Extension	72°C	5 min

¹ 20-30 seconds/kb, increase as necessary.

4. After PCR, maintain the reaction at 4°C or store at -20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis.
6. Visualize by ethidium bromide or SafeView™ (Cat No. **G108**) staining.