



MegaFi™ One-Step RT-PCR

Cat. No. G597

Store at -20°C.

Product Description

MegaFi™ One-Step RT-PCR is an enzyme mix of OneScript® Hot Reverse Transcriptase and MegaFi™ 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. It 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". MegaFi™ One-Step RT-PCR 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 mix 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 components and briefly centrifuge.
3. Thermocycling conditions for standard PCR:

Step	Temperature	Duration	Cycle(s)
cDNA Synthesis	60°C	15 min	1
Initial Denaturation	98°C	30 sec	1
Denaturation	98°C	5-10 sec	25 - 35
Annealing	50-72°C	10-30 sec	
Extension	72°C	20-30 sec/kb ¹	
Final Extension	72°C	2 min	1
Holding	4°C	-	1

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.