LeGene Premium One-Step RT-PCR

Catalog No. 6800-05: LeGene Premium One-Step RT-PCR, Size: 50 reactions
Catalog No. 6800-10: LeGene Premium One-Step RT-PCR, Size: 100 reactions
Catalog No. 6800-25: LeGene Premium One-Step RT-PCR, Size: 250 reactions

Store at -20°C
LeGene Premium One-Step RT-PCR is stable for 2 years when stored at -20°C (non-frost-free).

Description
LeGene Premium One-Step RT-PCR is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA in a single tube. The proprietary 2X Reaction Mix contains optimized buffer including dNTPs, Mg^{2+}, enhancer, and stabilizer. This system uses a mixture of an engineered MMLV RT, RnaUsScript-20 Reverse Transcriptase (RNaseH minus), and DnaUs Hot Start *Taq* DNA polymerase in an optimized reaction buffer and can detect RNA targets up to 3 kb.

DnaUs Hot Start *Taq* DNA polymerase is an antibody-inactivated hot-start enzyme designed to block polymerase activity between ambient to RT reaction temperature. RnaUsScript RT enzyme can synthesize cDNA at a temperature range of 40-55^{o}C. Once the PCR step reaches denaturation temperature (94^{o}C), *Taq* DNA polymerase activity is restored and the resulting PCR exhibits higher sensitivity, specificity and yield. The amount of input total RNA can range from 1 pg to 2 µg. Sufficient reagents are provided for 50, 100, or 250 amplification reactions of 50 µl each.

Features
- Convenient one-tube setup
- Broad range of RT reaction temperature (40-55^{o}C)
- Hot start PCR system
- Fully optimized RT-PCR buffer and robustness
- High sensitivity, specificity, and reproducibility

Kit components

	6800-05 **50 Rxns**	6800-10 **100 Rxns**	6800-25 **250 Rxns**
• Enzyme mix: RnaUsScript RT/DnaUs Hot Start *Taq* Mix	50 µl	100 µl	250 µl
• 2X Reaction mix (optimized buffer contains dNTP and Mg^{2+})	1.25 ml	2X1.25 ml	5X1.25 ml

Product qualification
LeGene One-Step RT-PCR System is functionally tested for amplification of a 1,025 bp of β-actin mRNA using 10 pg of total HeLa RNA as a template.
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Recommended RT-PCR reaction assembly
The following protocol is suggested as a starting point.

Components	50 µl Rxn	Final Concentration
2X Reaction mix	25 µl	1X
Enzyme mix	1.0 µl	
Forward Primer (10 µM)	1.0 µl	200 nM
Reverse Primer (10 µM)	1.0 µl	200 nM
RNA template	x µl	Variable (0.1 pg - 2 µg)
Final volume with distilled water	to 50 µl	

Note: *Efficient cDNA synthesis can be achieved between 10-30 minutes of incubation at 40-55°C. We recommend 30 minutes of incubation at 45°C as a general starting point.*

1. Assemble the reaction on ice.
2. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification.

   cDNA synthesis: 1 cycle: 45°C for 30 min

   Denaturation: 1 cycle: 94°C for 2 min

   PCR amplification: 40 cycles:
   94°C for 15 s
   55-65°C for 30 s
   72°C for 1 min/kb

3. Analyze RT-PCR amplified products by gel electrophoresis.

Limitations of Use
For research use only. Not for use in diagnostic procedures.