

## LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System

Catalog No. 6220-02: LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System, Size: 20 reactions

Catalog No. 6220-05: LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System, Size: 50 reactions

### Store at -20°C

LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System is stable for 2 years when stored at -20°C (non-frost-free).

### Description

LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System is optimized to synthesize high yield cDNA from total or poly(A)<sup>+</sup> purified RNA. The system can be used with a broad range of RNA template concentrations, from as low as 100 ng to as high as 20 µg of total RNA. LeGene 1<sup>st</sup> Strand cDNA Maxi Archive reverse transcriptase is a mixture of RnaUs MMLV RT and a ribonuclease inhibitor protein. It is ideally suited for high throughput expression profile studies using real-time quantitation as well as end-point RT-PCR. The synthesized cDNA can be used for short-term and long-term archival storage, quantitative PCR, and conversion to cRNA. The system contains all the components needed for first-strand cDNA synthesis. Reagents are sufficient for 20 or 50 cDNA synthesis reactions of 100 µl reaction volume.

### Components

	6220-02 <b>(20 Rxn)</b>	6220-05 <b>(50 Rxn)</b>
• RnaUs MMLV RT and RNase inhibitor mix	20 µl	50 µl
• 2X cDNA Synthesis Buffer (contains all necessary components including Mg <sup>2+</sup> , dNTPs, and random hexamer)	1.0 ml	1.25 ml X2
• Nuclease-free water	1.0 ml	1.25 ml X2

### Product Qualification

LeGene 1<sup>st</sup> Strand cDNA Maxi Archive System is functionally tested for a target amplification of 350 bp β-actin mRNA from a 10<sup>6</sup>-fold diluted cDNA sample of 10 µg total RNA input.

### 1<sup>st</sup> Strand cDNA Synthesis Protocol

1. Prepare the following RT reaction mixture on ice.

Components	1X Rxn
2X cDNA Synthesis Buffer	50 µl
RNA (100 ng - 20 µg)	x µl
RnaUs MMLV RT and RNase inhibitor Mix	1.0 µl
Nuclease-free water	to 100 µl
Total	100 µl

2. Mix gently and then centrifuge 3 seconds to collect contents.

3. Incubate at room temperature (22-24°C) for 10 minutes, and then transfer the tubes to 37 °C and incubate for 90 minutes.

*Note: RT temperature may be optimized between 37 and 42 °C.*

3. Inactivate RT by heating at 85°C for 5 min.

4. Use 1-2  $\mu$ l of the first strand reaction out of 100  $\mu$ l for PCR amplification. Store at 4 °C for short-term (up to 48 hours) or at -20 °C for long-term.

### Helpful guidelines

*No RT-control:* For an accurate gene expression profile study by RT-qPCR (especially rare copy gene quantitation), it is important to include a "no RT" control reaction in the experimental design.

For example, if you start with 1  $\mu$ g of total RNA for cDNA synthesis, use 1  $\mu$ g of total RNA as a template for the no RT control. Any signal from the no RT control reaction may be attributed to genomic DNA contamination.

*DNA digestion of RNA:* If amplification products are detected from the PCR reaction in the absence of reverse transcriptase, it may be necessary to remove residual genomic DNA from the RNA sample. Assemble the reaction, incubate at room temperature for 10 minutes, add EDTA (final conc 2.5 mM), and then inactivate DNase I by incubation at 65°C for 15 minutes. This mixture can be directly used for cDNA synthesis.

Components	Rxn
Total RNA (1-5 $\mu$ g)	x $\mu$ l
10X Reaction Buffer (200 mM Tris-Cl(pH 8.4), 500 mM KCl, 20 mM MgCl <sub>2</sub> )	1 $\mu$ l
DNaseI (RNase-free grade)	1 unit
Nuclease-free water	to 10 $\mu$ l

### Additional Products

- Catalog No. 6100-10: RnaUs MMLV Reverse Transcriptase, Size: 5,000 units
- Catalog No. 6100-50: RnaUs MMLV Reverse Transcriptase, Size: 20,000 units
- Catalog No. 6150-05: RnaUsScript Reverse Transcriptase, Size: 5,000 units
- Catalog No. 6150-20: RnaUsScript Reverse Transcriptase, Size: 20,000 units
- Catalog No. 6200-02: LeGene 1<sup>st</sup> Strand cDNA Synthesis System, Size: 20 reactions
- Catalog No. 6200-05: LeGene 1<sup>st</sup> Strand cDNA Synthesis System, Size: 50 reactions
- Catalog No. 6200-10: LeGene 1<sup>st</sup> Strand cDNA Synthesis System, Size: 100 reactions
- Catalog No. 6210-05: LeGene Express 1<sup>st</sup> Strand cDNA Synthesis System, Size: 50 reactions
- Catalog No. 6210-20: LeGene Express 1<sup>st</sup> Strand cDNA Synthesis System, Size: 200 reactions
- Catalog No. 6250-05: LeGene Premium Express 1<sup>st</sup> Strand cDNA Synthesis System, Size: 50 Rxn
- Catalog No. 6250-20: LeGene Premium Express 1<sup>st</sup> Strand cDNA Synthesis System, Size: 200 Rxn
- Catalog No. 6300-05: LeGene One-Step RT-PCR, Size: 50 reactions
- Catalog No. 6300-10: LeGene One-Step RT-PCR, Size: 100 reactions
- Catalog No. 6300-25: LeGene One-Step RT-PCR, Size: 250 reactions

### Limitations of Use

*For research use only. Not for use in diagnostic procedures.*