Product Description

Script Max First-Strand cDNA Synthesis Kit can be used for synthesis of fragmented or full-length first-strand cDNA. After second-strand cDNA synthesis, the products can be used for high-throughput sequencing library construction.

Components

Script Max 1st Strand cDNA Synthesis Kit is supplied in the following package configurations.

Components	BR3N701-03 (24 T)	BR3N701-06 (96 T)
5× Max RT Buffer	96 μL	384 μL
Super HS RTnase (NA)	48 μL	192 μL

Storage

Store at -20±5℃.

Notes

1. Mix thoroughly before use.
2. Use RNase-free consumables and clean the experimental area regularly.
3. Other reagents required for library construction should be prepared by the user or purchased separately.
4. For Research Use Only.

Protocol

1. RNA Pre-denaturation (Optional) [a]

1.1 Thaw Random Primer at room temperature or on ice. Mix thoroughly by inversion or gentle tapping, briefly centrifuge, and keep on ice before use. Prepare the reaction mixture according to Table 1.

Table 1. RNA Pre-denaturation Reaction Setup

Component	Volume (μL)
Random Primer	2
RNA [b]	12
Total	14

Note a: This step is optional for RNA samples with complex secondary structures. Simple RNA samples or fragmented RNA samples can be used directly for first-strand synthesis after adding the reaction components, without RNA pre-denaturation.

Note b: Add the total amount of RNA according to experimental requirements. If the volume is less than 12 μL, add nuclease-free water to 12 μL.

1.2 Mix gently by pipetting, then briefly centrifuge to collect the reaction mixture at the bottom of the tube. Run the following program on a thermal cycler (heated lid: 80℃): 70℃for 5 min; immediately place on ice for 3 min.

2. First-strand cDNA Synthesis

2.1 Remove the first-strand synthesis reagents from -20℃, thaw at room temperature or on ice, mix thoroughly by inversion, briefly centrifuge to collect liquid from the tube wall, and keep on ice before use. Prepare the first-strand cDNA synthesis reaction mixture according to Table 2.

Table 2. First-strand cDNA Synthesis Reaction Setup

Component	Volume (μL)
(Denatured) RNA [a]	14
5× Max RT Buffer	4
Super HS RTnase (NA)	2
Total	20

Note a: If RNA has not been pre-denatured, add 2 μL Random Primer and an RNA sample with a total volume of 12 μL.

2.2 Mix gently by pipetting, then briefly centrifuge to collect the reaction mixture at the bottom of the tube. Set the thermal cycler according to Table 3 and start the reaction.

Table 3. First-strand cDNA Synthesis Program

Step	Temperature	Time
1	25℃	3 min
2	50℃	15-30 min
3	75℃	15 min
4	4℃	Hold

2.3 The products can be used directly for second-strand cDNA synthesis or gene-expression analysis, or stored temporarily at -20℃. For long-term storage, keep at -80℃.