艾科瑞生物
Accurate Biology

Instruction for Use

Evo M-MLV Plus 1st Strand cDNA Synthesis Kit

AG11615
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is optimized for 1st Strand cDNA synthesis from RNA template using Reverse Transcriptase. This product uses enhanced and optimized *Evo M-MLV* Plus Reverse Transcriptase, with superior cDNA elongation capability. This product can inhibit non-specific binding and extension of Reverse Transcriptase to achieve good specificity and integrity of full strand cDNA. This product is suitable for cDNA library construction and full strand cDNA synthesis.

2. Kit Information

Kit Name	Cat. No	Specification
Evo M-MLV Plus 1st Strand cDNA Synthesis Kit	AG 11615	50 rxns / 20 µl

3. Transportation and Storage

Storage	Store at −20°C
Transportation	Transport at −20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
Evo M-MLV Plus RTase (200U/µl)	50 µl
5X RTase Plus Reaction Buffer	200 µl
RNase Inhibitor (40U/µl)	25 µl
dNTP Mix (10mM each)	50 µl
Oligo dT (18T) Primer (50 µM)	50 µl
Random 6 mers Primer (50 µM)	100 µl
RNase Free Water	1 ml

Note: Must thaw and thoroughly mix, then centrifuge and collect all reagents to bottom of tubes before use.

Note: If precipitation occurs for the 5X RTase Plus Reaction Buffer, fully dissolve precipitation before use.

Note: Recommend to conduct all reagent preparation steps on ice.

5. Protocol

5.1 RNA Template Denaturation and Annealing

(1) Prepare the RNA Template mix as below

Components	Input Volume
Oligo dT (18T) Primer (50 µM)	1 µl
Or Random 6 mers Primer (50 µM)	1 µl (0.4-2 µl) *1
dNTP Mix (10mM each)	1 µl
Template RNA *2	-
RNase free water	Up to 10 µl

*1: Recommended Random 6 mers Primer (50 µM) input is 1 µl; Input volume could be altered per experimental condition. If synthesized cDNA is used for downstream qPCR (Real Time PCR) experiment, input shall be 1-2 µl.

*2: Recommended Total RNA Input volume is ≤5 µg; mRNA input is ≤1 µg.

(2) Run Thermal Cycling Program as below to denature and anneal the RNA Template.

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Temperature	Time
65°C	5 min
4°C	-

5.2 1st Strand cDNA Synthesis

(1) Prepare the cDNA Synthesis reaction mix as below

Components	Input Volume
Mix from Step 5.1	10 µl
dNTP Mix (10mM each)	4 µl
RNase Inhibitor (40U/µl)	0.5 µl (20U)
Evo M-MLV Plus RTase (200U/µl)	1 µl (200U)
RNase free water	Up to 20 µl

(2) Run Thermal Cycling Program as below.

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Temperature	Time
30°C*1	10 min
42°C*2	30-60 min*3
95°C	5 min*4
4°C	-

*1: This step of thermal cycling program is mandatory for experiment using Random 6 mer Primers. It could be skipped for experiment using other RT primers.

*2: Extension temperature could be optimized to 50 °C to improve reaction specificity, if non-specific amplification occurs or unsatisfactory specificity.

*3: For normal condition, 30 min of extension is recommended; it could be prolonged up to 60 min for large target fragment or complicated structure.

*4: For large target fragment, to sustain integrity of 1st Strand cDNA, this step could be altered to 70°C, 15 min.

5.3 PCR Amplification and Further Analysis

Retrieved cDNA from above steps could be used for further analysis and experiment of PCR.

Recommended cDNA input volume should be less than 1/10 of the total PCR reaction system volume.

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