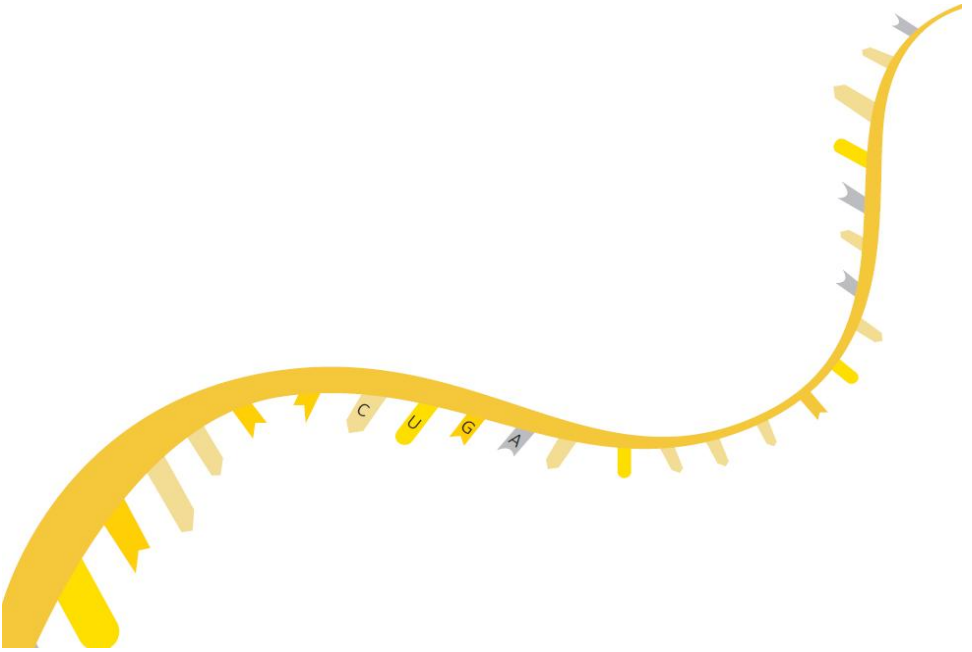
ABclonal First Strand Synthesis Module

RK20353



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1. Introduction

ABclonal First Strand Synthesis Module (RK20353) is suitable for RNA fragmentation and first strand cDNA synthesis in RNA-seq library construction. The first strand cDNA product can be directly used for subsequent second strand cDNA synthesis without purification. The RT Reagent in this module does not contain Actinomycin D and can be used with the ABclonal Second Strand Synthesis Module (RK20346) to make RNA libraries.

Note: RNA fragmentation relies upon the Mg^{2+} concentration of the Frag / Elute Buffer; to generated correctly fragmented product, input RNA samples should avoid containing additional metal ions, such as Mg^{2+}, or metal ion chelators, such as EDTA.

2. List of Components

Table 1. Kit Contents

	Tube name	24RXN	96RXN	500RXN
●	2X Frag / Elute Buffer	144 µL	576 µL	1.5 mL X 2
●	RT Reagent	192 µL	768 µL	4 mL
●	First Strand Synthesis Enzyme Mix	48 µL	192 µL	1 mL

3. Storage

All components should be stored at - 20℃.

4. Additional Materials Required

Poly(A) RNA enrichment: ABclonal Poly(A) mRNA Purification Module (RK20341).

rRNA depletion: ABclonal rRNA Depletion Module (H / M / R) (RK20348).

Other Materials: Nuclease-Free Water, Vortex mixer, Low absorption EP tubes, PCR tubes, thermocycler

5. Protocol

Protocol A for use with Total RNA

If the input sample is total RNA, the ABclonal Poly(A) mRNA Purification Module (RK20341) or the ABclonal rRNA Depletion Module (H/M/R) (RK20348) are recommended for purification. Add 1X Frag/Elute buffer to the magnetic beads for RNA elution and fragmentation.

Note: 1X Frag/Elute buffer can be obtained by diluting 2X Frag/Elute Buffer with equal volume of nuclease-free water.

Protocol B for use with Purified RNA

If the input sample is purified Poly(A)-mRNA or rRNA-depletion RNA, the 2X Frag / Elute Buffer provided by this module can be used to directly fragment the purified RNA, which can be immediately used in the first strand cDNA synthesis reaction.

Step 1. RNA Fragmentation

1.1 Prepare the purified poly(A)-RNA or rRNA-depleted RNA according to the following table:

Table 2. Fragmentation Reaction Setup (per sample)

	Component	Volume
	Purified RNA	5 μL
●	2×Frag / Elute Buffer	4 μL
	Total volume	**10 μL**

1.2 Mix the solution thoroughly by pipetting it up and down.

1.3 Place the PCR tube on the thermocycler and follow the reaction procedure described in Table 3.

Table 3. Fragmentation Reaction Program

Expected-insert size	Fragmentation time
200-300 nt	94℃ 15 min，4℃ hold
300-450 nt	94℃ 10 min，4℃ hold
400-700 nt	94℃ 5 min，4℃ hold

Step 2. First Strand cDNA Synthesis

2.1 Thaw the RT Reagent at room temperature, then prepare the following reaction on ice:

Table 4. First Strand Synthesis Reaction Setup (per sample)

	Component	Volume
	Fragmented RNA	10 μL
●	RT Reagent	8 μL
●	First Strand Synthesis Enzyme Mix	2 μL
	Total volume	**20 μL**

2.2 Mix the solution thoroughly by pipetting it up and down.

2.3 Place the PCR tube on the thermocycler and follow the reaction procedure described in Table 5.

Table 5. First Strand Synthesis Reaction Program

Temperature	Time
25℃	10 min
42℃	15 min
70℃	15 min
4℃	Hold

2.4 Immediately perform second strand cDNA synthesis using the ABclonal Second Strand Synthesis Module (RK20346).

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