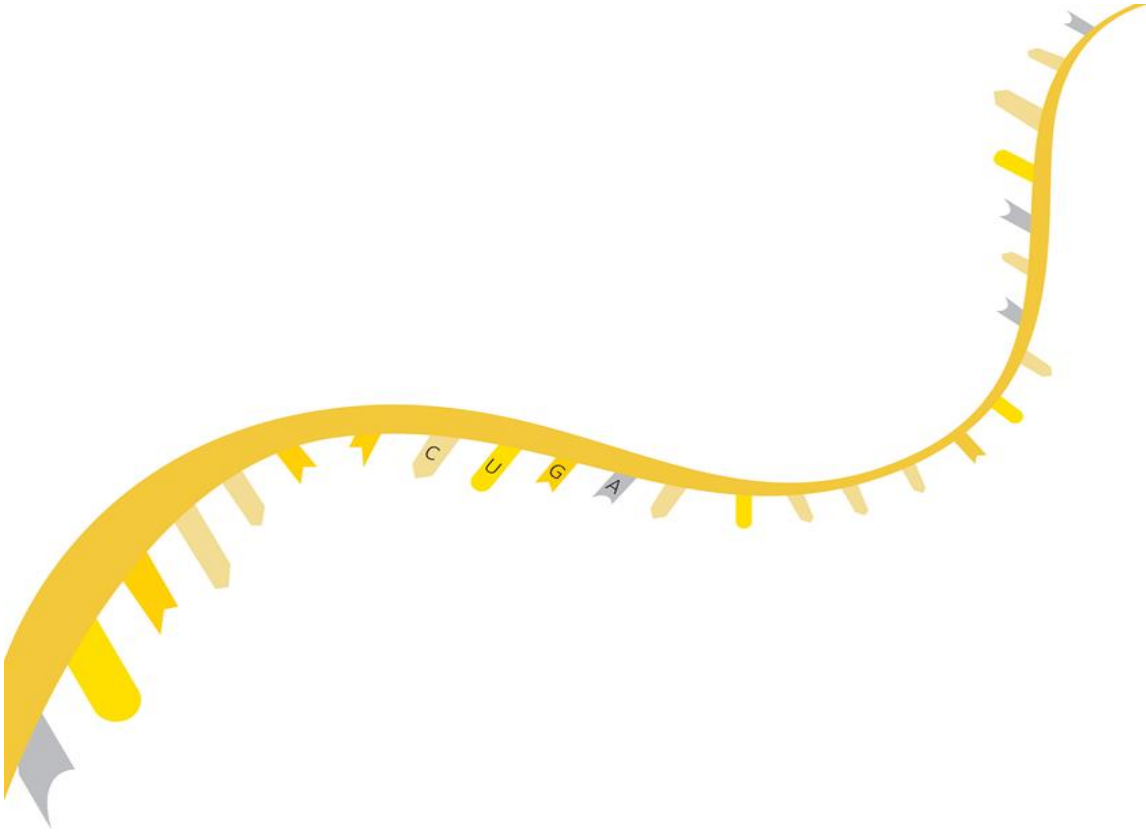
2X Frag/Elute Buffer ®

RM20225



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Version: N17 H14v1.1

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1. Product Overview

- 2X Frag/Elute Buffer (ABclonal, Cat. RM20225) was used for RNA Fragmentation and First-strand systhesis steps in RNA library construction.
- The kit is applicable to total RNA samples or purified mRNA samples from eukaryotes, including animals, plants, and fungi.
- 2X Frag/Elute Buffer contains Mg2+ and interrupts the RNA by high temperature, so the RNA samples used for fragmentation cannot contain metal ion components such as mg2+ and EDTA.
- 2X Frag/Elute Buffer contains Random Primer as reverse transcription primer in the First strand cDNA synthesis step.

2. Kit Components

Tube Name	96 RXN
2X Frag/Elute Buffer	528 µL

3. Storage

- Storage: -20°C to -10°C.
- Long-distance transportation: The kit should be transported with dry ice or with both dry ice and ice bags at -40°C to -20°C

4. Protocol

1. RNA Fragmentation

1.1 Prepare the Prepare the RNA fragmentation system according to the following table:

Reagent	Volume
Input RNA	10-1000 ng
2X Frag/Elute Buffer	6 μL
Nuclease-free Water	Up to 12 μL

1.2 If the Input RNA does not need to be fragmented, 2. First strand cDNA synthesis was performed directly; If the Input RNA needs to be fragmented, please perform the RNA fragmentation procedure according to the recommended conditions in the table below (heating lid temperature 105°C):

Target Fragment Size	Fragmentation Condition
200-300 nt	94°C 15 min, 4°C hold
300-450 nt	94°C 10 min, 4°C hold
400-700 nt	94°C 5 min, 4°C hold

1.3 After cooling to 4°C, take out the tube, centrifuge it instantaneously, and place the tube on the magnetic rack until the solution becomes clear. Transfer 10 μL of the supernatant into another PCR tube, and immediately use it for the first strand cDNA synthesis.

2. First Strand cDNA Synthesis

2.1 Thaw RT Reagent at room temperature, and prepare the following system on ice:

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

* : The pre-mix can be prepared in advance, and its volume should be 1.1 times the sample volume to make up for the natural loss.

** : The 2X Frag/Elute Buffer contains the Random Primer required for the First strand cDNA synthesis. Ensure that the 2X Frag/Elute Buffer is added when performing this step.

2.2 Mix the prepared system well by pipetting, centrifuge it instantaneously, and incubate it in the PCR system (heating lid temperature 105°C).

Temperature	Time
25°C	10 min
42°C	15 min
70°C	15 min
4°C	Hold

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