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NGS RNA First Strand Synthesis Module

Catalog Number: E117-1, E117-2

Table 1. Kit Components and Storage

Kit Component	E117-1 (25 rxn)	E117-2 (100 rxn)	Storage	Stability
Random Primers	25 µL	100 µL	-20 °C, avoid repeated free-thaw	The product is stable for 12 months when stored as directed.
First Strand Synthesis Reaction Buffer (5×)	100 µL	400 µL		
First Strand Synthesis Enzyme Mix	25 µL	100 µL		
Nuclease-free Water	250 µL	1 mL		

Product Description

The NGS First Strand Synthesis Module contains the enzymes and buffers required to convert RNA into cDNA using random priming. The fast, user-friendly workflow has minimal hands-on time and is compatible with upstream poly(A) mRNA enrichment and rRNA depletion methods; it is also compatible with downstream 2nd strand cDNA synthesis for RNA-seq workflows.

Applications

- cDNA synthesis for RNA library preparation and sequencing.

Protocol

1. In a nuclease-free 0.2 mL PCR tube, add 10 µL of fragmented mRNA and 1 µL of Random Primers.
2. Incubate in a preheated thermocycler for 5 minutes at 65°C with heated lid set to 100°C. Hold at 4°C.
3. Spin tube briefly and place on ice.
4. To the fragmented mRNA and Random Primers add:

First Strand Synthesis Reaction Buffer	4 µL
First Strand Synthesis Enzyme Mix	1 µL
Nuclease-free Water	4 µL
Total volumes	20 µL

5. Mix by pipetting gently up and down.
6. Incubate the samples in a preheated thermal cycler (with the heated lid set to 100°C):
   10 minutes at 25°C
   30 minutes at 68°C
   Hold at 4°C
7. Place the tube on ice.
8. Proceed directly to second strand synthesis using NGS RNA Second Strand Synthesis Module (Cat no. E118).