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NGS RNA Fragmentation Module

Catalog Number: E115

Table 1. Kit Components and Storage

Kit Component	Unit Size (200 rxn)	Storage	Stability
RNA Fragmentation Buffer (10×)	400 µL	RT	The product is stable for 36 months when stored as directed.
RNA Fragmentation Stop Solution (10×)	400 µL		

Product Description

The NGS RNA Fragmentation Module is optimized to fragment RNA into small pieces using divalent cations under elevated temperature.

Applications

- RNA fragmentation.

RNA Fragmentation Protocol

This protocol is used to fragment 10-400 ng of rRNA-depleted, or poly(A)-enriched RNA.

1. Mix the following components in a microcentrifuge tube:

Purified RNA	10-400 ng
RNA Fragmentation Buffer (10×)	2 µL
Nuclease-free water	to 20 µL
Total volume	20 µL

2. Place the tubes in a thermocycler and carry out the fragmentation as follows:

Input RNA	Desired mean library insert size (bp)	Fragmentation
Intact	100-200	8 min at 94°C
	200-300	6 min at 94°C
	300-400	6 min at 85°C
Partially degraded	100-300	1-6 min at 85°C
Degraded	100-200	30 sec at 65°C

3. Place the tubes on ice and proceed immediately to 1st strand synthesis. Or add 2 µL of RNA Fragmentation Stop Solution (10×), and proceed RNA clean up.

Clean Up Fragmented RNA Using Ethanol Precipitation

1. Mix the following components in a microcentrifuge tube:

Fragmented RNA from Step 3	22 µL
3 M Sodium Acetate, pH 5.2	2 µL

Linear Acryamide, 10 mg/mL	1 µL
100% Ethanol	60 µL
Total volume	85 µL

2. Incubate at -80°C for 30 minutes.
3. Centrifuge at 14,000 rpm for 25 minutes at 4°C in a microcentrifuge.
4. Carefully remove ethanol.
5. Wash pellet with 300 µL of 70% ethanol.
6. Centrifuge and carefully remove 70% ethanol.
7. Air dry pellet for up to 10 minutes at room temperature to remove residual ethanol
8. Resuspend in 20 µL Nuclease-free Water.