NEBNext® Magnesium RNA Fragmentation Module

NEB #E6150S

200 reactions

Version 4.0_2/20

Table of Contents

The Module Includes

The volumes provided are sufficient for preparation of up to 200 reactions (NEB #E6150S). All reagents should be stored at –20°C.

NEBNext RNA Fragmentation Buffer (10X)

NEBNext RNA Fragmentation Stop Solution (10X)

Required Materials Not Included

- RNA Cleanup Kit (Monarch RNA Cleanup Kit, NEB #T2030)

or

- 3M Sodium Acetate, pH 5.2
- 100% Ethanol
- 70% Ethanol
- Linear Acrylamide 10 mg/ml

Description

The NEBNext Magnesium RNA Fragmentation Module has been optimized to fragment RNA into small pieces using divalent cations under elevated temperature.

The NEBNext Magnesium RNA Fragmentation Module contains buffers that are suited for RNA fragmentation. Each of these components must pass rigorous quality control listed on each individual component page.

Lot Control: The lots provided in the NEBNext Magnesium RNA Fragmentation Module are managed separately and are qualified by additional functional validation. Individual reagents meet stringent criteria in the additional quality controls listed on each individual component page.

Functionally Validated: The NEBNext Magnesium RNA Fragmentation Module is functionally validated through construction of a transcriptome library using the NEBNext mRNA sample preparation reagents and sequencing on an Illumina® sequencing instrument.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Applications

RNA Fragmentation

NEBNext Magnesium RNA Fragmentation Module Protocol

Starting Material
Total RNA (2–50 µg) or purified mRNA (50–250 ng)

1. Mix the following components in a sterile PCR tube:

COMPONENT	VOLUME (µl)
Purified RNA	1–18
RNA Fragmentation Buffer (10X)	2
Nuclease-Free Water	variable
Total Volume	20

2. Incubate in a preheated thermal cycler for 1–5 minutes at 94°C.*
3. Transfer tube to ice.
4. Add 2 µl 10X RNA Fragmentation Stop Solution.

   * Fragmentation time should be adjusted depending on amount and type of RNA and desired sizes of fragments (See Figure 1).

Clean Up Fragmented RNA Using the Monarch RNA Cleanup Kit (NEB #T2030)

1. Add 78 µl of the Nuclease-Free Water to the 22 µl fragmented RNA from Step 4. Purify sample using the Monarch® RNA Cleanup Kit (NEB #T2030) following manufacturer instructions. Elute in 14.5 µl Nuclease-Free Water. The recovered volume should be ~13.5 µl.

   Note: column purification removes short RNA Fragments and enriches the sample for RNA fragments longer than 200 nucleotides.

Alternatively, Clean Up Fragmented RNA Using Ethanol Precipitation

1. Mix the following components in a sterile 1.5 ml microcentrifuge tube:

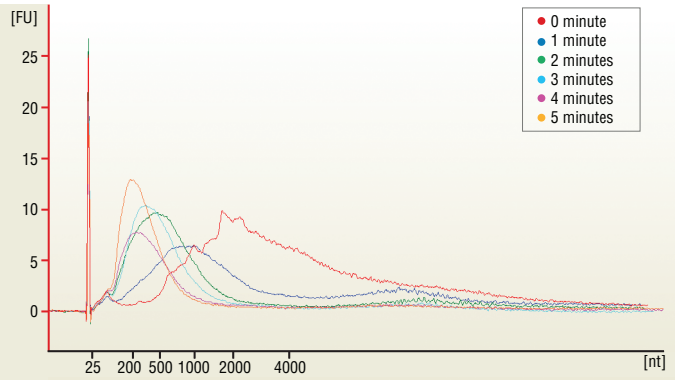
COMPONENT	VOLUME (µl)
Fragmented RNA from Step 4	22
3M Sodium Acetate, pH 5.2	2
Linear Acrylamide, 10 mg/ml	1–2
100% Ethanol	60
Total Volume	85–86

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 13.5 µl Nuclease-free Water.

Assess the Yield and the Size Distribution of the Fragmented RNA

Take 1 µl of the fragmented RNA and dilute it 1:10 with nuclease-free water. Run 1 µl in the Agilent Bioanalyzer® 2100 using a RNA Pico chip (Figure 1).

Figure 1: Relative size distribution of eukaryotic mRNA fragments as seen using the Bioanalyzer 2100.



Poly (A)⁺ mRNA (40 ng) purified from High Quality Universal Human Reference RNA Agilent 740000 was fragmented in 1X NEBNext Magnesium RNA Fragmentation Buffer for 1–5 minutes at 94°C. Fragmentation Reaction was stopped in 1X NEBNext Fragmentation Stop Solution. Samples were diluted 1:10 in Nuclease-Free Water and analyzed in the Bioanalyzer 2100.

Kit Components

NEB #E6150S Table of Components

NEB #	PRODUCT	VOLUME
E6186A	NEBNext RNA Fragmentation Buffer	0.4 ml
E6187A	NEBNext RNA Fragmentation Stop Solution	0.4 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	7/11
2.0	Create “Kit Component – Table of Components” for small and large size kits. Delete individual component information pages.	5/18
3.0	Update “Required Materials not included,” Update module protocol.	5/19
4.0	Apply new manual format	2/20