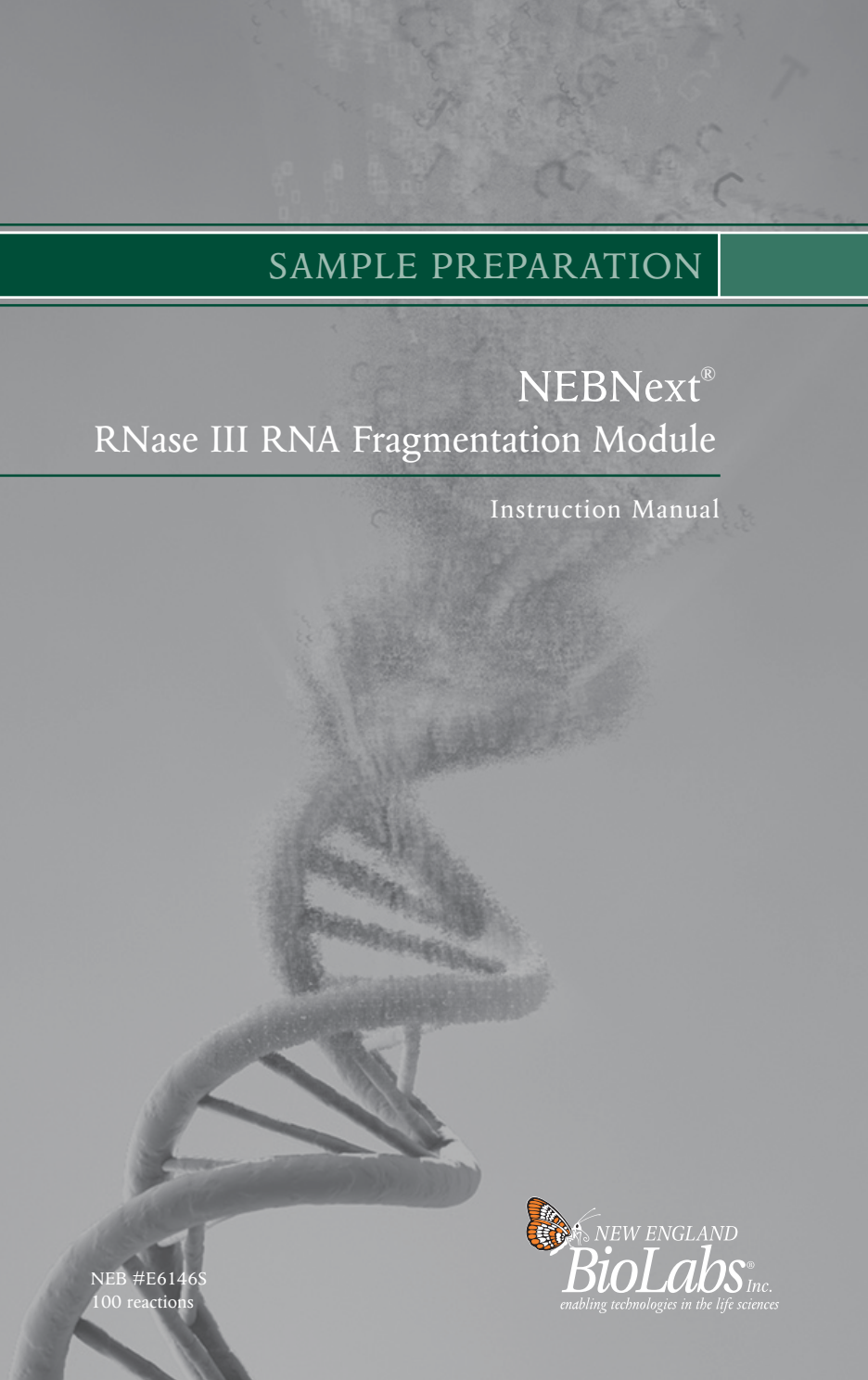
SAMPLE PREPARATION

NEBNext®
RNase III RNA Fragmentation Module

Instruction Manual



NEB #E6146S
100 reactions

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enabling technologies in the life sciences

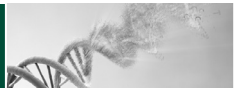


Table of Contents:

The Module Includes:

The volumes provided are sufficient for preparation of up to 100 reactions (NEB #E6146S). (All reagents should be stored at –20°C):

NEBNext® RNase III

NEBNext® RNase III Reaction Buffer (10X)

Required Materials Not Included:

RNeasy MinElute Cleanup Kit (Qiagen # 74204)
or
3M Sodium Acetate, pH 5.2
100% Ethanol
70% Ethanol
Linear Acrylamide 10 mg/ml

Description:

The NEBNext® RNase III RNA Fragmentation Module has been optimized to fragment RNA into small pieces using RNase III enzyme. RNase III is an endonuclease enzyme that cleaves long double-stranded RNA into short fragments containing 5´-PO_4 and 3´-OH ends.

The NEBNext RNase III RNA Fragmentation Module contains enzyme and buffer that are suited for RNA fragmentation. Each of these components must pass rigorous quality control standards listed on each individual component page

Lot Control: The lots provided in the RNase III RNA Fragmentation Module are managed separately and are qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays listed on each individual component page.

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® RNase III RNA Fragmentation Module Protocol

Starting Material: Purified mRNA (50–250 nanograms)

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

	Volume (µl)
Purified mRNA (100 nanograms)	variable
RNase III (1 unit/µl)	1
RNase III Reaction Buffer (10X)	2
Nuclease-Free Water	variable
total volume	20

2. Incubate in a preheated thermal cycler for 5 minutes at 37°C.*
3. Add 80 µl of cold Nuclease-Free water.
4. Transfer tube to ice.
5. Clean up RNA fragments using a RNA column purification kit or ethanol precipitation.

*These conditions have been optimized to fragment 100 nanograms of mammalian mRNA into RNA fragments with a normal distribution with a mean peak at 200 nucleotides (see figure 1). Other types of RNA (Plant, Bacterial, Yeast) or other amounts of mammalian mRNA might require adjusting the amount of enzyme and/or incubation time depending on the RNA fragment size desired.

Clean Up Fragmented RNA Using RNeasy MinElute Spin Columns

1. Purify sample using RNeasy MinElute Cleanup Kit (Qiagen #74204) following the manufacturer's instructions. Elute with 14 µl nuclease-free water. The recovered volume should be ~12 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:

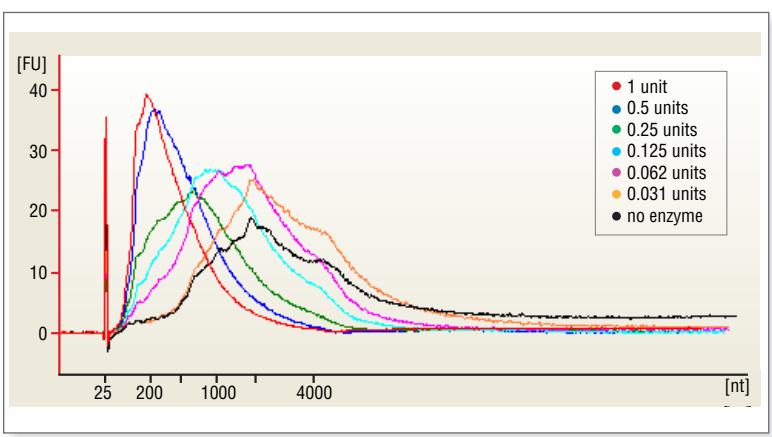
	Volume (µl)
Fragmented RNA from Step 4	100
3M Sodium Acetate, pH 5.2	10
Linear Acrylamide, 10 mg/ml	1–2
100% Ethanol	300
total volume	411–412

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% freshly prepared 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 appropriate volume of Nuclease-Free Water.

Assess the Yield and the Size Distribution of the Purified RNA Fragments.

Run 1 µl of the purified RNA fragments 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 (100 nanograms) purified from the Universal Human Reference Total RNA (Stratagene) was fragmented in 1X NEBNext RNase III RNA Reaction Buffer for 5 minutes at 37°C using different amounts of the NEBNext RNase III enzyme (1; 0.5; 0.25; 0.125; 0.062; 0.031 units). RNA fragments were column purified and analyzed on the Bioanalyzer 2100.

NEBNext® RNase III:

#E6171A: 0.1 ml **Concentration: 1 unit/µl**

Store at –20°C

Source:
An *E. coli* strain containing the *E. coli* RNase III gene

Quality Control Assays

SDS-PAGE Purity: RNase III is > 95% pure as determined by SDS PAGE.

16-Hour Incubation: 50 µl reactions containing RNase III and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing RNase III and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis

Endonuclease Activity: Incubation of a 50 µl reaction containing RNase III with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in less than 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

Exonuclease Activity (Radioactive Release): Incubation of a 50 µl reaction containing RNase III with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

RNase Activity: Incubation of a 10 µl reaction containing RNase III with 40 ng of a single-stranded RNA transcript for 16 hours at 37°C results in no detectable degradation of RNA as determined by gel electrophoresis.

Phosphatase Activity: Incubation of RNase III in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Functional Activity: One unit of RNase III is defined as the amount of enzyme catalyzing the cleavage of 2 micrograms of 500 bp double-stranded RNA substrate to approximately 12–30 bp fragments in 15 minutes at 37°C in 1X RNase III Reaction Buffer.

Lot Controlled

NEBNext® RNase III Reaction Buffer:

#E6172A: 0.2 ml **Concentration: 10X**

Store at –20°C

1X NEBNext RNase III Reaction Buffer:
10 mM Tris-HCl
10 mM $Mg(Cl)_2$
1 mM DTT
60 mM NaCl
pH 8.3 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing 1X RNase III Reaction Buffer and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing 1X RNase III Reaction Buffer and 1 µg of T3 DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction containing 1X RNase III Reaction Buffer with 1 µg of ϕX174 RF I supercoiled DNA for 4 hours at 37°C results in less than 10% conversion to RF II (nicked molecules) as determined by gel electrophoresis.

RNase Activity: Incubation of a 10 µl reaction containing 1X RNase III Reaction Buffer with 40 ng of a single-stranded RNA transcript for 16 hours at 37°C results in no detectable degradation of RNA as determined by gel electrophoresis.

Phosphatase Activity: Incubation of 1X RNase III Reaction Buffer in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM p-nitrophenyl phosphate at 37°C for 4 hours yields no detectable p-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

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