

RNase III

(*E.coli* carrying plasmid with *rnc* gene of *E.coli* RNase III)

Cat. No.	size
E1340-01	200 units
E1340-02	1 000 units

Concentration:

2 U/μl

Unit Definition:

One unit of RNase III, *E. coli*, is the amount of enzyme required to digest 1 μg of dsRNA to siRNA in 20 minutes at 37°C in a total reaction volume of 50 μl.

Storage Conditions:

Store at -20°C.

References:

1. Yang, D. et al. (2002) *Proc. Natl. Acad. Sci. USA*, 99, 9942-9947.
2. Calegari, F. et al. (2002) *Proc. Natl. Acad. Sci. USA*, 99, 14236-14240.
3. Donze, O. and Picard, D. (2002) *Nucleic Acids Res*, 30, e46. Laboratory, Cold Spring Harbour.
4. Morlighem, J.E. et al. (2007) *Biotechniques*, 42, 599-606.
5. Evguenieva-Hackenberg, E. and Klug, G. (2000) *J. Bacteriol.* 182, 4719.
6. Nicholson, A. (1999) *FEMS Microbiol. Rev.* 23, 371.
7. Drider, D. et al. (1999) *J. Mol. Microbiol. Biotechnol.* 1, 337.
8. Grunberg-Manago, M. (1999) *Annual Rev. Genet.* 33, 193.

RNase III is a divalent metal dependent nuclease that cleaves long double-stranded RNA (dsRNA) into short dsRNAs (13-30 bases). Products of RNase III mimic siRNA structures produced by Dicer enzyme (5'-PO₄, 3'-OH and a dinucleotide 3' overhang). This unique feature enable to generate a population of RNAs that, after transfection into mammalian cells, can induce RNAi (1-4).

Applications:

- Digestion of long dsRNA to short dsRNA.
- RNA structure studies (5).
- Transfection of RNase III cleavage products can be used to induce RNAi in mammalian cells.
- RNA processing and maturation studies (6-8).

10 x Reaction Buffer:

300 mM Tris-HCl, 1.6 M NaCl, 10 mM dithiothreitol, 1 mM EDTA, pH 8.0 at 25°C.

Storage Buffer:

30 mM Tris-HCl, 500 mM NaCl, 1 mM dithiothreitol, 0.5 mM EDTA, 50% glycerol, pH 8.0 at 25°C.

Quality Control:

All preparations are assayed for contaminating exonuclease, endonuclease, for nonspecific RNase and single- and double-stranded DNase activities. Typical preparations are greater than 90 % pure, as judged by SDS polyacrylamide gel electrophoresis.

Reagent supplied:

COMPONENT:	E1340-01	E1340-02
10 x Reaction Buffer	750 μl	2 x 1.5 ml
MnCl ₂ 0.5 M	200 μl	1 ml
RNase III 2 U/μl	100 μl	500 μl
RNase-free Water	1 ml	3 x 1.5 ml

Example Reaction:

1. Assemble reaction on ice as follows:
2. Incubate for 30-60 min at 37°C.
3. RNase III may sediment so mix gently enzyme before use without centrifugation.

Component:	Amount:
10 x Reaction Buffer	5 μl
MnCl ₂ (final concentration in reaction 20 mM)	2 μl
Substrate RNA	1-4 μg
RNase III	2-4 U
RNase-free Water	to 50 μl

4. If cleavage products are used for transfection of mammalian cells, we recommend using EURx Universal RNA/miRNA Purification kit (Cat. No E3599) for purification of short RNA fragments in high quality.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, tel. +48 58 524 06 97, fax +48 58 341 74 23

Method for generation of gene-specific dsRNA as RNase III substrate

The EURx T7 Transcription Kit (Cat. No. E0901) is suitable for generating gene-specific dsRNA as a substrate for RNase III cleavage. Here is a general, brief outline of the procedure, which can be further adapted to meet any specific experimental requirements:

1. **PCR amplification:** Amplify the gene of interest. Extend both 5'-end of gene specific primers with T7 promoter sequences.

T7 RNA Polymerase – Primer Design:

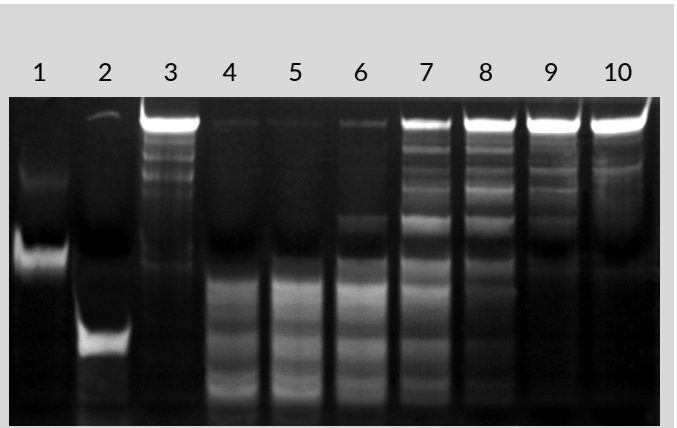
|Recognition | > Transcription Start
|-17 -5| -1|
5'- T AAT ACG ACT CAC TAT A -3'

The four nucleotides marked in blue are variable, but either should be A or T. T7 RNA Polymerase requires an extension of the primer 5 to 6 bp upstream (5'-) of the T7 promoter. The first nucleotides following the 3' end of the promoter should be GG, AG or GA. Make sure, the gene specific primer portion will anneal to the target DNA template at temperatures well above 55°C. Here is an example for a widely used, known-to-work 5'-terminal primer extension for introducing a T7 promoter sequence:

5'-GAA ATT AAT ACG ACT CAC TAT AGG-sequence
specific primer part -3'

2. **DNA Template Purification:** Purify amplified DNA using EURx PCR/DNA Clean-Up DNA Purification Kit (Cat. No. E3520) or EURx Agarose-Out DNA Purification Kit (Cat. No. E3540) when template DNA is excised from agarose gel. Procedure of purification gives yields approx. 20- 25 µg of high-quality, RNase-free template DNA for T7 RNA transcription.
3. **T7 RNA Transcription:** Transcribe RNA with the EURx T7 Transcription Kit (Cat. No. E0901) according to the instructions supplied along with the kit. 1-2 µg of purified, RNase-free(!) DNA are required for a 25 µl reaction volume.

Note: Successful RNA transcription following simultaneous initiation of T7 transcription from both 5'-ends of DNA is possible. T7 RNA polymerases starting transcription from opposite ends of the same DNA strand will not interfere with each other, when coming across during RNA elongation.



RNase III digestion of dsRNA.

Lane 1—dsDNA fragment 25 bp,

Lane 2—dsDNA fragment 16 bp,

Lane 3—uncleaved substrate, 1.5 µg of pre-tRNA for glycine from *T. thermophilus*,

Lanes 4–10 varying amounts of RNase III: 6 U, 3 U, 1.5 U, 0.75 U, 0.37 U, 0.18 U, 0.09 U respectively.

Cleavage was performed for 20 min at 37°C and analyzed on 20% TBE polyacrylamide gel stained with ethidium bromide.

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www.eurx.com.pl, orders@eurx.com.pl, tel. +48 58 524 06 97, fax +48 58 341 74 23