RNase III

(*Escherichia coli*)

RNase III
(Escherichia coli)

RNase III is a divalent metal dependent nuclease that cleaves long double-stranded RNA (dsRNA) into short dsRNAs (13-30 bases). Products of RNaseIII mimic siRNA structures produced by Dicer enzyme (5'-PO_4, 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).

Cat. No.	Size
E1340-01	200 units
E1340-02	1000 units

Unit Definition:
One unit of RNaseIII, *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

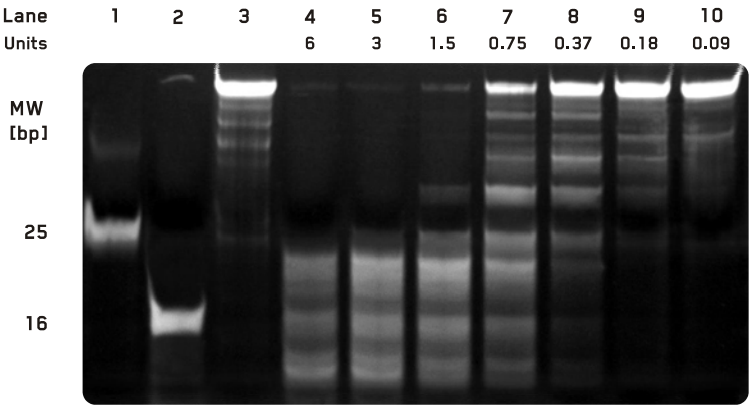
Source:
E.coli strain carrying a plasmid with the rnc gene of *E.coli* RNase III.

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, 5 99-606.*
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.*

Description:

- → 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).



RNase III digestion of dsRNA. 1- ds DNA fragment 25bp, 2-dsDNA fragment 16bp, 3-uncleaved substrate, 1.5 ug of pretRNA for glycine from *T.thermophilus*, 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 performed for 20 min at 37˚C and analyzed on a 20% [w/v] TBE polyacrylamide gel stained with ethidium bromide.

Storage Buffer:

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

Reaction Buffer:

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

Package Contents:

Reagent supplied	E1340-01	E1340-02
10 x Reaction Buffer	750 µl	2 x 1.5 ml
$MnCl_2$ 0.5 M	200 µl	1.0 ml
RNase III, 2 U/µl	100 µl	500 µl
RNase-free water	1.0 ml	3 x 1.5 ml

Quality Control:

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

RNase III

DIGESTION PROTOCOL

Preparation of RNaseIII Digestion:

Assemble reaction on ice as follows:

Component	Volume/Reaction	Final Concentration
10 x Reaction Buffer	5 µl	1x
$MnCl_2$	2 µl	20 mM
Substrate RNA	1-4 µg	20-80 ng/µl
RNaseIII, 2 U/µl	1-2 µl	2-4 U
H_2O, nuclease free	@ 50 µl	-
Total Volume	**50 µl**	-

Incubate 30-60 minutes at 37˚C.

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.

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 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 promotor 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 (marked with green font). The first nucleotides following the 3' end of the promotor should be GG, AG or GA (marked with red font). 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 introduction of 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 excising template DNA from agarose gels. Typically, these purification procedures yield 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.