RNase III, *E. coli*

Store at –20°C
Do not store in a frost-free freezer.

Catalog #:	AM2290
Amount:	250 Units
Source:	An *E. coli* strain overexpressing recombinant RNase III.
Volume:	250 µL
Unit Concentration:	1 U/µL
Unit Definition:	One unit is defined as the amount of enzyme catalyzing the cleavage of 1 µg of 500 bp dsRNA substrate to approximately 12–30 bp fragments in 60 min at 37°C.
Unit Assay Conditions:	50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT (pH 7.9).
Additional Materials Included:	500 µL 10X RNase III Reaction Buffer 500 mM NaCl 100 mM Tris pH 7.9 100 mM $MgCl_2$ 10 mM DTT
Storage Conditions:	Store at –20°C. ***Do not store in a frost-free freezer.***
Storage Buffer:	(Not included) 500 mM NaCl, 20 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.5 mM DTT and 50% Glycerol.

USER INFORMATION

Product Description:

Ambion recombinant bacterial RNase III (25.6 KDa) is a homodimeric, divalent metal ion dependent nuclease. RNase III cleaves long double-stranded RNA (dsRNA) into short 12–30 base dsRNAs containing a 5'-PO4, 3'-OH, and a dinucleotide 3' overhang—the same structures found in siRNA produced by Dicer enzyme. Introduction of RNase III cleavage products can induce RNAi in mammalian cells [1]. Cleavage of long dsRNA by RNase III will generate a complex mixture of siRNAs, several of which may be capable of inducing RNAi. The amount of siRNA generated by RNase III cleavage will depend on the size of the long dsRNA that is digested and the size range of the siRNAs generated. We have found that complete digestion of long dsRNA molecules by RNase III into 12–15 base fragments yields siRNAs that are capable of inducing RNAi in mammalian cells.

Applications:

siRNA Preparation Using *E. coli* RNase III

Prepare Long dsRNA Corresponding to the Gene of Interest
One of the following strategies should be used to produce dsRNA: (1) Prepare a single dsDNA template with opposing phage RNA polymerase promoters (e.g., T7) at the 5' ends of each strand. Use the template in a single in vitro transcription reaction to synthesize dsRNA. (2) Use two DNA templates that are identical except that a phage RNA polymerase promoter sits at opposite ends of the region to be transcribed and transcribe the two templates either in the same or in separate in vitro transcription reactions. We recommend using the MEGAscript® RNAi Kit (Cat #AM1626) for preparation of dsRNA because the kit provides all the required methods and reagents to produce high quality long dsRNA using either of the strategies mentioned above. It also includes reagents to purify the dsRNA after synthesis. For more information about the MEGAscript RNAi Kit and access to the detailed Instruction Manual that describes the protocol and transcription template design, see www.ambion.com/catalog/CatNum.php?1626.

Digest dsRNA with RNase III

1. Mix 15 µg of long dsRNA with 5 µL 10X RNase III Reaction Buffer, 15 µL RNase III (15 U), and Nuclease-free Water (Cat #AM9915G) to a final volume of 50 µL. (The reaction is scalable.)
2. Incubate at 37°C for 1 hr.
3. Purify siRNA by running the product over a Microcon-30 filter (Millipore, Cat #42409) as recommended by the manufacturer. Collect the flow-through that contains the dsRNA smaller than 30 bp.

The concentration of the dsRNA can be determined by measuring the absorbance at 260 nm using a spectrophotometer.

Transfection and Analysis of siRNA effect
The siRNAs generated by RNase III can be transfected into cells at a final concentration of 10–150 nM using an appropriate transfection agent. We recommend the siPORT™ *Amine* or siPORT™ *NeoFX*™ Transfection Agents, both of which are included in the Ambion *Silencer*® Transfection II Kit (Cat #AM1631). For more information about the *Silencer* siRNA Transfection II Kit, including transfection protocols and troubleshooting advice, see www.ambion.com/catalog/CatNum.php?1631. The siRNA effect can be assessed at both the RNA and protein levels using a variety of methods, including Northern analysis, RT-PCR, Western analysis, and immunofluorescence.

References:

1. Yang D, Buchholz, F, Huang Z, Goga A, Chen C-Y, Brodsky FM, and Bishop MJ (2002). Short RNA duplexes produced by hydrolysis with *Escherichia coli* RNase III mediate effective RNA interference in mammalian cells. *PNAS* **99(15)**: 9942–9947.

QUALITY CONTROL

RNase III is rigorously tested for contaminating protease activity. Functionality is determined in a unit activity assay.

OTHER INFORMATION

Material Safety Data Sheets:

Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Warranty and Liability:

For research use only. Not for use in diagnostic procedures.

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