ShortCut® RNase III

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M0245S 0041211114111

M0245S

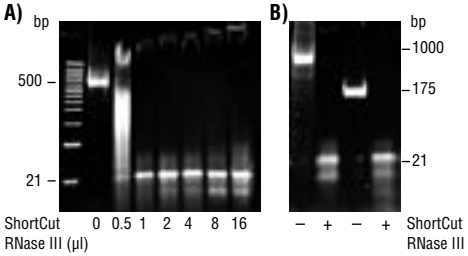
RR 37°

200 units	**2,000 U/ml**	**Lot: 0041211**
RECOMBINANT	**Store at –20°C**	**Exp: 11/14**

Description: ShortCut® RNase III, used with its manganese-containing reaction buffer, converts long double-stranded RNA into a heterogeneous mix of short (18–25 bp) interfering RNAs (siRNA) suitable for RNA interference in mammalian cells (1–3). 1.5 units (1 µl) of ShortCut RNase III is sufficient to convert 1 µg of dsRNA into siRNA suitable for RNA interference in mammalian cells.

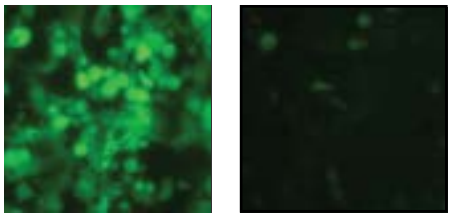
Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase III gene (rnc) and the gene coding for maltose binding protein (MBP).

Applications:
- Gene silencing
- Target validation



ShortCut RNase III digestion of dsRNA: (A) Varying amounts of ShortCut RNase III were incubated with 2 µg of a 500 bp dsRNA for 20 minutes at 37°C in a 50 µl reaction. Digests were analyzed by 20% TBE polyacrylamide electrophoresis. Marker lane contains a mixture of 21 bp siRNA Marker and 100 bp DNA Ladder (NEB #N3231). (B) dsRNA fragments (1 kb and 175 bp) were digested with ShortCut RNase III. Digests were analyzed by 20% TBE polyacrylamide gel electrophoresis.

More Units, Higher Concentration



GFP Silencing in COS-7 Cells: COS-7 cells co-transfected in a 24 well plate with a plasmid expressing GFP in the absence (control) or the presence of 30 ng (4 nM) of GFP siRNA prepared using the ShortCut RNAi Kit. Cells were photographed 48 hours post-transfection.

Supplied in: 500 mM NaCl, 10 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

Reagents Supplied with Enzyme:
10X ShortCut Reaction Buffer
10X $MnCl_2$ (200 mM)
10X EDTA (500 mM)
RNase-free Glycogen (10 µg/µl)

Reaction Conditions: 1X ShortCut Reaction Buffer. Supplement with 20 mM $MnCl_2$ (supplied). Incubate at 37°C.

1X ShortCut Reaction Buffer:
50 mM Tris-HCl
1 mM DTT
50 mM NaCl
pH 7.5 @ 25°C

Unit Definition: One unit 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.

ShortCut RNase III Digestion Protocol:

1. Synthesize, quantify and purify the desired fragment of dsRNA to be digested. ShortCut RNAi Kit (NEB #E2450) and HiScribe T7 *In Vitro* Transcription Kit (NEB #E2030) provide reagents and protocols to synthesize long dsRNA.
2. Combine the following, in order:

dH_2O	70 µl – x µl
10X ShortCut Reaction Buffer	10 µl
dsRNA	x µl (10 µg)
ShortCut RNase III	10 µl
10X $MnCl_2$	10 µl
Total Volume	100 µl

3. Mix and incubate for 20 minutes at 37°C.
4. Add 10 µl 10X EDTA to stop the reaction. ***Do not heat inactivate.*** *Heat inactivation reduces the yield of siRNA.*

Purification of siRNA by Ethanol Precipitation Protocol:

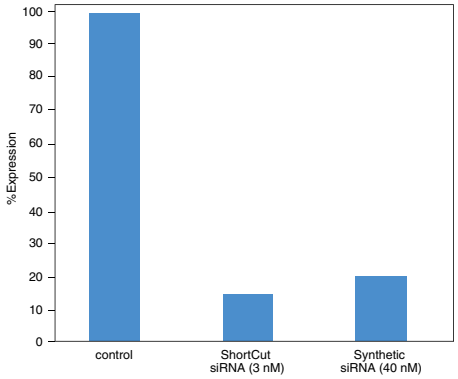
Ethanol precipitation efficiently removes unwanted components from the ShortCut digestion and quantitatively yields the siRNA in a small pellet.

1. Add one-tenth volume of 3 M NaOAc (pH 5.5), 2 µl RNase-free Glycogen and 3 volumes of cold 95% ethanol. Place at –70°C for 30 minutes or –20°C for 2 hours.
2. Spin for 15 minutes in a microcentrifuge at 14,000 rpm.
3. Remove supernatant; to the pellet, add two volumes 80% ethanol, incubate at room temperature for 10 minutes, centrifuge for 5 minutes, decant the tube.
4. Allow the pellet to air-dry.
5. Dissolve the dried RNA in 10 mM Tris-HCl (pH 7.0), 1 mM EDTA, or dH_2O to approximately 1/3 volume of the original ShortCut digestion.

   The siRNA solution can be quantitated by UV absorbance, comparison on agarose gel to DNA standards or fluorescence.

Transfection Guidelines: After ethanol precipitation, the resuspended siRNA mixture is ready for transfection in mammalian cells using reagents and protocols suitable for oligonucleotide transfections. Alternatively, calcium phosphate or electroporation may be used; both have been reported to be efficient in transfecting short RNAs (4).

A very small amount of siRNA is sufficient for effective silencing as compared to single sequence siRNAs(1). We recommend testing 25–100 ng of siRNA per transfection well (24 well format) as a starting point. In this format, these amounts correspond to approximately 5–15 nM.



Comparison of ShortCut siRNA vs synthetic siRNA: Silencing of luciferase in human embryonic kidney cells (HEK-293) using siRNA mixture corresponding to firefly luciferase prepared using the ShortCut RNAi Kit. The effect of silencing with 25 ng (3 nM) of ShortCut siRNA compared with 300 ng (40 nM) of a published synthetic siRNA, is expressed as the relative expression of firefly luciferase over renilla luciferase activities measured using a Dual-Luciferase® Reporter Assay System (Promega).

Frequently Asked Questions:

- *The siRNA yield from my ShortCut RNase III digestion is lower than expected.*

  The problem may be over-digestion. Make sure to use the recommended concentration of dsRNA and ShortCut RNase III. It is possible that your dsRNA quantitation is not accurate. Repeat quantitation or perform a small scale titration of ShortCut RNase III with a given amount of dsRNA (see figure). You can then increase both proportionally. Do not use reaction times over 30 minutes since this will decrease the yield of siRNA.

- *Can I use the ShortCut RNase III digestion directly for transfection since there is no undigested long dsRNA present?*

  This is not recommended. The digestion reaction components interfere with many transfection protocols; so, the ethanol precipitation step is necessary.

- *Can fewer units of ShortCut RNase III be used with longer incubation times to generate siRNA?*

  No. The concentration of ShortCut RNase III and reaction time have been optimized for use with the maganese-containing reaction buffer to produce the maxium yield of siRNA.

References:

1. Morlighem, J.E. et al. (2007) *Biotechniques* 42, 599–606.
2. Yang, D. et al. (2002) *Proc. Natl. Acad. Sci. USA* 99, 9942–9947.
3. Calegari, F. et al. (2002) *Proc. Natl. Acad. Sci. USA* 99, 14236–14240.
4. Donzé, O. and Picard, D. (2002) *Nucleic Acids Res.* 30, e46.

Companion Products:

Biotinylated T7 Primer (25-mer)
#S1273S 0.5 A_{260} units

LITMUS 28i Vector
#N3528S 20 µg

LITMUS 38i Vector
#N3538S 20 µg

Magnetic Separation Rack
#S1506S

Streptavidin Magnetic Beads
#S1420S 5.0 ml

CERTIFICATE OF ANALYSIS