HEN1 miRNA Methyltransferase

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0228S 001111213121

M0228S

NEB2 RR SAM 37° Yes

1,000 units **50,000 U/ml** **Lot: 0011112**

RECOMBINANT **Store at –20°C** **Exp: 12/13**

Description: HEN1 is a plant miRNA methyltransferase that methylates the terminal ribose in short double-stranded RNAs. In *Arabidopsis*, 2´-O-methylation of the 3´ ribose of miRNAs and siRNAs is mediated by HEN1 (1, 2). In HEN1 mutants, small RNAs were found unmethylated with a 3´ poly (U) tail, suggesting 3´ end methylation protects small RNAs from uridylation (3). Purified HEN1 methylates both miRNA/miRNA and siRNA/siRNA duplexes with a preference for 21–24 nucleotide RNA duplexes with 2 nucleotide overhangs (4). HEN1 does not methylate single-stranded RNA or DNA.

Source: An *E. coli* strain that carries the cloned HEN1 from *Arabidopsis*.

Applications:

- 3´ end labeling of siRNA and miRNA

Supplied in: 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2,
S-adenosylmethionine (32 mM).

Reaction Conditions: 1X NEBuffer 2, 128 µM S-adenosylmethionine and 100–500 ng miRNA duplex in 25 µl.
Incubate at 37°C.

1X NEBuffer 2:
10 mM Tris-HCl
50 mM NaCl
1 mM DTT
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to transfer 1 pmol of methyl group to a miRNA duplex in 10 minutes at 37°C.

Quality Control Assays

RNase Assay: Incubation of a 10 µl reaction containing 50 units of HEN1 miRNA Methyltransferase with 40 ng RNA (a 0.3 kb *in vitro* transcript) for 2 hours at 37°C, resulted in no detectable degredation of the RNA as determined by denaturing PAGE analysis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 50 units of HEN1 miRNA Methyltransferase with 1 µg sonicated ^{3}H DNA (10^5 cpm/µg) for 4 hours at 37°C released < 0.5% of the total radioactivity.

Endonuclease Activity: Incubation of a 10 µl reaction containing 50 units of HEN1 miRNA Methyltranferase with 300 ng pUC19 Plasmid DNA for 4 hours at 37°C resulted in < 10% conversion from supercoiled to nicked molecules as determined by agarose gel electrophoresis.

Storage of SAM: S-adenosylmethionine or SAM is stored at –20°C as a 32 mM solution dissolved in 0.005 M sulfuric acid and 10% ethanol. Under these conditions SAM is stable for up to 6 months. SAM is unstable at (pH 7.5), 37°C, and should be replenished in reactions incubated longer than 4 hours.

Heat Inactivation: 70°C for 10 minutes

Protocol for a Typical miRNA Methylation Reaction

Combine the following components in sterile microfuge tube:

10X NEBuffer	2.5 µl
3.2 mM SAM*	1 µl
miRNA duplex (100–500 ng)	X
H_2O	X
RNase inhibitor (optional)	20 units
HEN 1 RNA Methyltransferase (50 units/µl)	1 µl
Total reaction volume	25 µl

Incubate at 37°C for 1 hour.

Notes:

Up to 500 ng miRNA duplex or 75 pmol 3´ end can be fully methylated in a 25 µl reaction.

SAM should be freshly diluted 1:10 with H_2O prior to use.

For miRNA isotope labeling, use 2 µl [^{14}C] SAM instead of cold SAM; miRNA amount can be reduced to increase specific activity.

(see other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

References:

1. Park, W., et al. (2002) *Curr. Biol.* 12, 1484–1495.
2. Yu, B., et al. (2005) *Science*, 307, 932–935.
3. Li, J., et al. (2005) *Curr. Biol.* 15, 1501–1507.
4. Yang, Z., et al. (2006) *Nucleic Acids Res.* 34, 667–675.

Companion Products:

S-adenosylmethionine (SAM)	
#B9003S	0.5 ml
RNase Inhibitor	
#M0307S	2,000 units
#M0307L	10,000 units

References:

1. Park, W., et al. (2002) *Curr. Biol.* 12, 1484–1495.
2. Yu, B., et al. (2005) *Science*, 307, 932–935.
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RNase Inhibitor	
#M0307S	2,000 units
#M0307L	10,000 units

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