

PRODUCT SPECIFICATION

eRNase1, sequence-specific endoribonuclease

Size: 20 U
100 U
lyophilized or in storage buffer

Supplied with: 1 ml of 10x Reaction Buffer

Product description

eRNase1 (an engineered dsRNA Mini-III endoribonuclease from *Bacillus subtilis*) cleaves double-stranded RNA substrates in a sequence-specific manner. eRNase1 specifically recognizes and cleaves ACC[^]U sequence. eRNase1 can be used to produce one or more defined, functional dsRNA molecules by excision from longer precursors (e.g. transcripts).

Sequence specificity

5' ...**ACC**[▽]**U**...3'
3' ...**U**[▲]**GGA**...5'

Unit definition

One Unit (1 U) is defined as the amount of the enzyme required to cleave 10 pmol of dsRNA substrate containing a single ACCU motif, in 1h, at 37°C, in 15 µl of 1x Reaction Buffer.

Source

The recombinant His-tagged protein is purified from *E. coli* containing a recombinant plasmid harboring the modified *mrnC* gene. The enzyme purity was validated by SDS-PAGE with Coomassie Blue staining showing the level of purity >95% and the absence of contaminating proteins.

10x Reaction Buffer

1 M Tris-HCl pH 7.5, 500 mM NaCl,
100 mM MgCl₂, 10 mg/ml BSA

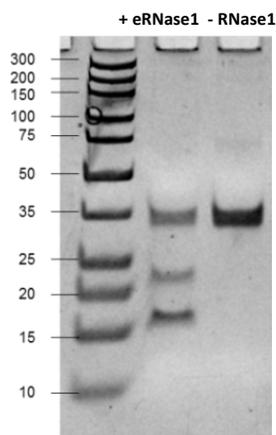
Recommended protocol for dsRNA cleavage

eRNase1 enzyme	0.5 - 1 U
dsRNA	10 pmol
10x Reaction Buffer	1.5 µL
RNase free water	up to 15 µL

Incubate at 37 °C for 1 h

The reaction is terminated by the addition of 1 µL of acid phenol solution (pH 4.5). Cleavage reaction may be scaled either up or down. The reaction products can be visualized by native polyacrylamide gel electrophoresis.

Cleavage of dsRNA substrate by eRNase1



The reactions were carried out for 1 h at 37°C, with or without 0.5 U of eRNase1, and 10 pmoles of substrate. Samples were resolved on 12% native polyacrylamide gel and the nucleic acids were visualized after staining with ethidium bromide.

Storage/Stability

Supplied in a storage buffer:

Store at -20°C. Avoid repeated freeze-thawing. Stable for at least 3 months with less than 10% loss in activity. The enzyme may be diluted in 1x Reaction Buffer for immediate use.

Supplied lyophilized:

Stable up to 2 weeks at room temperature. For long-term storage keep at -20°C. Before use

dissolve in a 50% glycerol_{aq} (water solution) to a final concentration of 0.5 U/ μ l and store at -20°C. Once dissolved in 50% glycerol_{aq} eRNase1 is stable for at least 3 months with less than 10% loss in activity.

Storage buffer

50 mM HEPES pH 7.5, 300 mM NaCl,
5 mM MgCl₂, 1 mM DTT, 50% glycerol

Product use limitation

This product is developed and designed exclusively for research purposes and *in vitro*

use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

References

Patents/pending patents no:

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