ambion
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RNase I

(Cloned)

Catalog Number AM2294, AM2295

Pub. No. 4393867 **Rev.** B

Contents	Cat. no.	Quantity	Storage conditions
RNase I, 100 U/µL	AM2294	10,000 Units	Store at -20°C. *Do not store in a frost-free freezer.*
	AM2295	25,000 Units	

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product description

RNase I is a highly purified preparation of *E. coli* ribonuclease I that degrades RNA with low sequence specificity.

Source: A *Pichia* strain overexpressing the *E. coli* RNase I gene (Meador and Kennell, 1990).

Unit definition: One unit is the amount of enzyme required to produce 1 µg of acid-soluble material from mouse liver RNA in 30 min at 37°C.

Materials not provided: Because of the variety of product applications, no reaction buffer is supplied.

Storage buffer: 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 50% glycerol (not included).

General Information

RNase I from *E. coli* was one of the first RNases to be characterized and was found to be completely nonspecific (Spahr and Hollingworth, 1961). The 27 kD endonuclease has been cloned and overexpressed in *E. coli* (Meador and Kennell, 1990). The commonly used RNases A and T1 are very specific in their degradation patterns and will cleave only after pyrimidines or guanidines, respectively. In marked contrast to these RNases, RNase I degrades all RNA dinucleotide bonds leaving a 5' hydroxyl and a 2', 3' cyclic monophosphate. It degrades any RNA to a mixture of mono-, di-, and trinucleotides and does not degrade DNA, although it does bind to DNA. It has a marked preference for single-stranded RNA over double-stranded RNA, which allows it to work well in RNase Protection Assays. It has a high specific activity which, coupled with its non-specificity, typically results in complete degradation of RNA using ng amounts of protein. RNase I is not inhibited by placental RNase inhibitor although, unlike RNases A and T1, it is easily inactivated by several different means (see below).

Although its function has not been clearly defined in vivo, there is sufficient evidence to speculate that it has dual functions. One function is to degrade RNA that is taken into the periplasm from the environment, and the other is to degrade stable RNA under certain conditions of stress. Under such conditions of stress, the RNase I that normally resides in the periplasmic space is allowed to enter the cytoplasmic space and degrade stable RNA (Beppu and Arima, 1969; Neu and Heppel, 1964; Ito and Ohnishi, 1983; Ono, *et al.*, 1987). These functions of RNase I are most likely the reason for its ability to easily degrade all 16 ribonucleotide bonds.

Ambion® cloned RNase I is greater than 99% pure, as judged by Coomassie-G staining of an SDS-PAGE gel. It does not require divalent cations and is fully active in Tris and phosphate buffers. It is 80% active in 0.3 M NaCl and 100% active in 0.1–0.2M NaCl. It is completely and irreversibly inactivated by 0.1% SDS or phenol extraction. It is inactivated by freeze-thaw cycles in aqueous buffers but is protected from inactivation by 20% glycerol.

Using RNase I

RNase I can be used in RNase Protection Assays using the reagents included in RPA III™ Kits (Cat. nos. AM1414, AM1415). It is the enzyme of choice for high resolution 3' and 5' mapping studies. In-house data shows that under the proper conditions it can degrade single-stranded RNA up to the point at which legitimately hybridized double-stranded RNA begins. Furthermore, it may be ideal for discriminating between transcripts for closely related gene families. For RNase Protection Assays using approximately 10 µg of total RNA

per sample, we suggest using 100–500 units of enzyme at 37°C for 30 min. For more information visit **www.lifetechnologies.com** > Technical Resources > Technical Reference Library > Ambion® Product Support > Ribonuclease Protection Assays.

RNase I also works well for eliminating RNA from DNA and/or protein preparations. For boiling lysate minipreps we suggest using 50 units at 37°C for 30 min.

Another application in which RNase I should work well is in RNase I footprinting reactions to determine the sequence specificity of an RNA binding protein. A direct result of the enzyme's lack of specificity is the generation of uniform ladders of partially degraded RNA probe. Since RNA can be labeled at the 5' end, as well as internally labeled, the region of protein binding can be easily determined.

Limited product warranty

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References

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