RNase R

Cat. No. E049

Store at -20°C.

Product Description

Rnase R is an *E.coli* exoribonuclease which **exhibits 3' to 5' exonuclease activity, efficiently digesting nearly all linear RNA species.** This enzyme does not digest circular, lariat, or double stranded RNA with short 3' overhangs (less than seven nucleotides). As such, this enzyme is ideally suited to the study of lariat RNA produced by traditional splicing, as well as circRNAs which arise through back-splicing. By removing linear RNAs from cellular or RNA extracts, RNase R great facilitates the identification of circular species through RNA-sequencing. This enables researchers to probe the landscape of splicing events with current depth of sequencing.

Product Component	Quantity	Part No.
RNase R	500 U (50 µl)	E049-1
10X RNase R Reaction Buffer	1.0 ml	E049-2

Protocol

1. Thoroughly thaw and mix individual components before use. Assemble reaction on ice:

Component	Volume (NGS)	Volume (non-NGS, e.g. qPCR)
Total RNA	at least 10 µg	1 - 10 µg
RNase R	4 µl + 4 µl (supplemented partway)	2 µl
10X RNase R Reaction Buffer	5 µl	2 µl
RNaseOFF Ribonuclease Inhibitor*	3 µl	0.5 µl
RNase-free ddH_2O	up to 50 µl	up to 20 µl

* Not included in kit. Purchase separately at **abm**, Cat. No. G138.

2. Gently mix the reaction components and briefly centrifuge.
3. For NGS applications, incubate the mixture at 37°C for 2 hours.
4. For non-NGS applications, incubate the mixture at 37 - 45°C for 2 - 3 hours.

General Notes

- For digestion of total RNA, longer incubations of 2 - 3 hours are often required.
- If degradation is inefficient, use a slightly higher incubation temperature (40-45°C) and supplement additional enzyme partway (e.g. 0.5 µl after 1 hour) through the procedure. The higher temperature is particularly useful for degrading highly structured linear RNAs, such as rRNAs. Do not exceed 45°C or incubate over 3 hours, as this may lead to non-enzymatic degradation.
- RNase R exhibits low activity on tRNA, rRNA and other highly structured RNAs, for which the 3' end is double stranded with a short 3' overhang. These RNA species can stall the enzyme and result in greatly reduced activity. In cases where inefficient degradation is observed, it is recommended to either upscale the digestion, use more RNase R, or remove rRNA from total RNA extracts prior to digestion.
- Keep in mind that circular RNAs represent a small proportion of total RNA (typical 0.1% - 0.01%), therefore RNase R treatment will most likely result in low levels of RNA (picogram-range), possibly undetectable by most methods. For this reason, a starting amount of at least 10 µg of total RNA is recommended for most downstream applications.
- While the enzyme can be heat inactivated, the procedure is not recommended since high heat can lead to RNA damage. Phenol-chloroform precipitation can be used instead. For NGS, solid phase reversible immobilization (SPRI) bead cleanup is recommended.
- Magnesium at concentrations of 0.1 - 1.0 mM is required for optimal activity. If EDTA is present, compensate by adding $MgCl_2$ to 1.0 mM final concentration.
- One unit is defined as the amount of RNase R that converts 1 µg of poly(A) into acid-soluble nucleotides in 10 minutes at 37°C.

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