

RNase R

Catalog
TBP0181

Unit
50 uL

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 greatly facilitates the identification of circular species through RNA-sequencing. This enables researchers to probe the landscape of splicing events with current depth.

Component

Product Component	Quantity
RNase R (10 U/μl)	50 μl
10X RNase R Reaction Buffer	1.0 ml

Store at -20°C.

Protocol

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice.

Component	Volume (NGS)	Volume (Non-NGS, e.g. qPCR)
Total RNA	at least 10 μg	Volume (Non-NGS, e.g. qPCR)
RNase R (10U/μl)	4 + 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 ₂ O	up to 50 μl	up to 20 μl

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 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 (pictogram-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 MgCl₂ to 1.0 mM final concentration.

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