RNase R

Cat.No.:HYJ412

Product Description

RNase R (Ribonuclease R) is a 3'-5' ribonuclease from the Escherichia coli RNR superfamily that cleaves RNA into dinucleotides and trinucleotides in a stepwise manner from the 3' to the 5' direction. RNase R digests nearly all linear RNA molecules but is less sensitive to circular RNA, lariat structures, or double-stranded RNA with 3' overhangs of less than 7 nucleotides. RNase R is commonly used in gene expression and alternative splicing studies. It digests linear RNA to enrich for circRNAs and lariat RNAs. RNase R cleaves linear RNA to enrich for circRNAs, which are used to construct intronic cDNA libraries from total RNA.

Components

Components	0.5 KU	5 KU
RNase R (20 U/μL)	25 uL	250 μL
10×Reaction Buffer	1 mL	10×1 mL

Product Information

Product Properties	Clear liquid
Source	E.coli
Activity Definition	One unit of activity (U) is defined as the amount of enzyme required to convert 1 μg of poly-r(A) into acid-soluble nucleotides in a standard reaction at 37°C within 10 minutes.
Optimum Reaction Temperature	37°C
Storage Buffer	50 mM Tris-HCl (pH 7.5 @ 25°C), 100 mM NaCl, 1mM DTT, 0.1mM EDTA, 50% Glycerol, 0.1% Triton® X-100
10×Reaction Buffer	200 mM Tris-HCl (pH8.20 @25°C)，1 mM $MgCl_2$，1 M KCl
Storage Conditions	-25 ~ -18°C, avoid repeated freezing and thawing

Quality Control

1. Purity (SDS-PAGE): ≥95%.
2. Residual *E. coli* DNA: RT-qPCR assay using 10 μL of RNase R for 16S rDNA with a Ct value of ≥35.
3. Endonuclease Activity: No degradation was detected when 1 μg of dsRNA was incubated at 37°C for 4 hours in a 20 μL reaction containing at least 10 U of RNase R.
4. DNase Activity: No degradation was detected when 1 μg of pUC19 DNA was incubated at 37°C for 4 hours in a 10 μL reaction containing at least 10 U of RNase R.
5. Functional Assay: RNase R functionality was tested in a reaction containing 10 U of RNase R and 3 μg of total RNA from 293T cells. RNA abundance was assessed by RT-qPCR. The ΔCt values of linear RNAs were ≥4.5, and those of circular RNAs were ≤1, calculated with and without RNase R digestion.

Reaction system and conditions

Components	Volume (20μL)	Volume (50μL)
10×Reaction Buffer	2 μL	5 μL
RNA	＜5 μg	＞5 μg
RNase R (20 U/μL)	1-3 U/μg RNA	1-3 U/μg RNA
Nuclease-free water	Up to 20 μL	Up to 50 μL

RNase R requires low (0.1-1.0 mM) magnesium concentrations for activity. Low EDTA concentrations in the RNA solution can negatively affect RNase R activity. A final concentration of $MgCl_2$ up to 1 mM can be used to compensate for EDTA in the substrate. Optimal activity is achieved at 37°C.

Reaction time: Incubate at 37°C for 15 minutes.

Reaction termination: Heat at 70°C for 10 minutes.

Purification and recovery

Digested RNA can be extracted using a phenol:chloroform:isoamyl alcohol (25:24:1, v:v) solution (or Trizol Reagent) and recovered by ethanol precipitation. Alternatively, RNA can be purified using RNA purification columns or magnetic beads.