RNase R, GMP Grade

Cat. No.: GMP-E224 animal-free, ampicillin-free

01/ Product Description

Ribonuclease R (RNase R), from the RNR superfamily of *E.coli*, is a Mg^{2+}-dependent 3'-5' exonuclease. RNA can be cleaved into dinucleotide and trinucleotide gradually from the 3'-5' direction by RNase R. RNase R can digest most of the linear RNA. However, it is difficult to digest circular RNA, lariat RNA and short double-stranded RNA molecules with less than 7 nucleotides of 3' end protrusion. RNase R is commonly used in gene expression and alternative splicing studies and digests linear RNA to enrich circular RNA or lariat RNA.

This product is obtained using recombinant protein technology. Our manufacturing processes are following the Pharmaceutical Manufacturing Guidelines. We guarantee the manufacturing and quality control comply with GMP regulation for tracking each and every step of the manufacturing process, including raw material sourcing.

02/ Quality Elements

Element	Standard
Appearance	Clear and transparent solution
Visible Particles	Meet the specification
pH	7.0-8.0
Activity	20U/μl
Purity	≥95%
Protein Content	Meet the specification
Endonuclease Residues	The degradation of substrate was ⩽10%
Exonuclease Residues	The degradation of substrate was ⩽10%
Other RNase Residues	$\|\Delta Ct\| = \|Ct_{\text{sample}} - Ct_{\text{positive control}}\| \leqslant 1$
Bacterial Endotoxin	<10EU/mg
Heavy Metal Residue	≤ 10ppm

03/ Complying to Following Regulations

1. ISO 9001:2015, certified facility.
2. GMP Appendix – Cellular therapeutic product National Medical Products Administration.
3. The Pandect of Genetic Therapeutic Product for Human Chinese Pharmacopoeia Commission 2022.
4. USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
5. USP Chapter <92>, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.
6. Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

04/ Application

1. Gene expression studies.
2. Alternative splicing study.

3. Enrichment of circRNA from biological samples.

4. Recognition of intronic lariat RNA.

5. Identification of exonic circRNA.

05/ Essential Product Information

Storage Buffer	50mM Tris-HCl, 100mM NaCl, 1mM DTT, 0.1% Triton® X-100, 0.1mM EDTA, 50% Glycerol (pH7.5 at 25°C)
Storage Conditions	Store at -20±5°C
Unit Definition	Under standard reaction conditions of 37°C, the enzyme required to convert 1μg poly(A) into acid-soluble nucleotides in 10 minutes is defined as 1U.

06/ Product Packaging

SKU	Size	Components	Volume
GMP-E224-U025	500 U	RNase R, GMP Grade (20U/μl)	25μl
GMP-E224-U250	5 KU	RNase R, GMP Grade (20U/μl)	250μl
GMP-E224-M001	20 KU	RNase R, GMP Grade (20U/μl)	1ml
GMP-E224-M010	200 KU	RNase R, GMP Grade (20U/μl)	10ml
GMP-E224-M050	1000 KU	RNase R, GMP Grade (20U/μl)	50ml

07/ Precautions

1) Clean the bench before the experiment, prepare the RNase-free materials, reduce or eliminate the RNase residual contamination such as solution, EP tube, and tip in the laboratory.

2) RNase R activity requires 0.1-1.0 mM Mg^{2+}.

3) With the increase of substrate RNA, the digestion time and enzyme usage can be appropriately increased.

4) The abundance of some circRNA or lariat RNA decreased after prolonged digestion of RNase R, possibly because of their weak digestion tolerance to RNase R.

5) EDTA can affect the activity of RNase R.

08/ Protocol

1. RNase R digestion experiment:

Components	Quantity
RNA sample	≤5μg
RNase R, GMP Grade (20U/μl)	2-4U/μg*
10× RNase R Buffer, GMP Grade	2μl
RNase Free Water	To 20μl

*1μg RNA sample need 2-4U RNase R

Digestion at 37 °C for 10-30min;

2. Inactivation step:

The enzyme could be inactivated by the reaction at 70°C for 5min (If the sample is purified and recovered later, this step can be omitted).

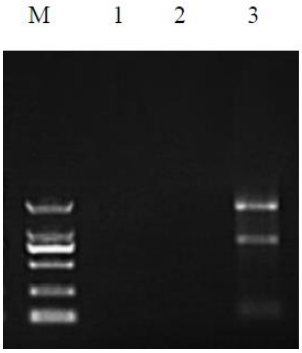
3. Purification and recovery:

The digested RNA was extracted using phenol: chloroform: isoamyl alcohol (25:24:1, V: V) solution and recovered by ethanol precipitation. RNA purification column or magnetic beads can be used for purification and recovery (If purification and recovery,

enzyme inactivation operation steps can be ignored).

09/ Applications Examples

1）Total RNA, ssRNA, and circRNA digestion experiments. 5U RNase R was mixed with total RNA and ssRNA, and 0.5 to 2U RNase R was mixed with circRNA, respectively. The mixture was incubated at 37℃ for 30min and 70℃ for 10min followed by electrophoresis.



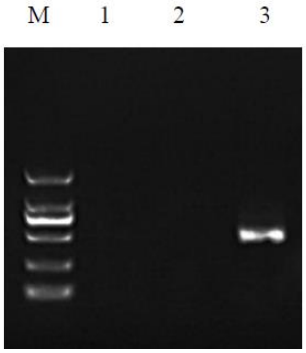
Total RNA Digestion

M: DNA Ladder.

Lane 1: Novoprotein RNase R.

Lane 2: Competitive brand RNase R.

Lane 3: Negative control.



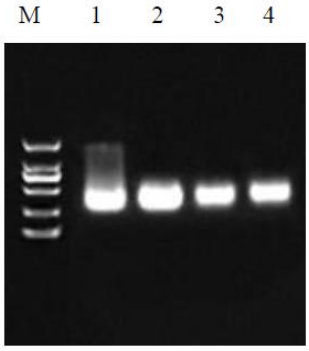
ssRNA Digestion

M: DNA Ladder.

Lane 1: Novoprotein RNase R.

Lane 2: Competitive brand RNase R.

Lane 3: Negative control.



circRNA Digestion

M：DNA Ladder.

Lane 1：Negative control.

Lane 2：0.5U RNase R incubated with 1μg circRNA.

Lane 3：1U RNase R incubated with 1μg circRNA.

Lane 4：2U RNase R incubated with 1μg circRNA.

10/ Related Products

Cat. No.	Product Name	Cat. No.	Product Name
GMP-E121	T7 RNA Polymerase, GMP Grade	GMP-E125	RNase Inhibitor, GMP Grade
GMP-E131	T7 RNA Transcription Enzyme Mix, GMP Grade	GMP-E127	DNase I, GMP Grade
GMP-M036	Pyrophosphatase, Inorganic (yeast), GMP Grade	GMP-S023-026	NTPs, GMP Grade
GMP-EB224	10×RNase R Buffer, GMP Grade		