RNase R

CATALOG NO.: M1228-500

AMOUNT: 500 U (50 µl)

CONCENTRATION: 10 U/µl

PRODUCT SOURCE: Recombinant *E.coli*

FORM: Liquid

KIT COMPONENTS:

Components	Volume	Part No.
RNase R (10 U/µl)	50 µl (500 U)	M1228-500-1
10X RNase R Reaction Buffer	1.0 ml	M1228-500-2

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 greater depth.

APPLICATIONS:

- Enriching circRNAs in biological samples
- Identification of intronic lariat sequences
- Identification of exonic circRNAs
- Studying alternative splicing
- Production of artificial circular RNAs

EZYME UNIT DEFINITION: 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.

ENZYME STORAGE BUFFER: 50 mM Tris-HCl (pH 7.5), 100mM NaCl, 0.1 mM EDTA, 1 mM DTT, and 50% (v/v) Glycerol

10X RNase R REACTION BUFFER COMPONENTS: 200 mM Tris-HCl, 1 M KCl, 1 mM $MgCl_2$, pH 7.5.

STORAGE CONDITIONS: Store at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for one year from the date of shipping when stored and handled properly.

SUGGESTED REACTION SETUP: *Note: May require optimization depending on the specific experiment.* Can be scaled up, according to the experiment needs. May not be effective on low concentrations of RNA (<0.1 ng/µL).

Components	NGS	Non-NGS (e.g. qPCR)
Total RNA	At least 10 µg	1-10 µg
RNaseR (20 U/µL)	2 + 2 µL (supplemented partway)	1 µL
RNaseR Buffer [10x]	5 µL	2 µL
ABM RNaseOFF (Cat# G138) [40 U/µL]	3 µL	0.5 µL
RNase-free ddH_2O	Fill up to volume	Fill up to volume
TOTAL VOLUME	**50 µL**	**20 µL**
Incubation Temperature	37°C	37-45°C
Duration	2 hours	2-3 hours

SAMPLE PROCESSING GUIDELINES AND TROUBLESHOOTING:

- 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 RNA 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. If 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 (typically 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 ETA is present, compensate by adding $MgCl_2$ to 1.0 Mm final concentration.

RELATED PRODUCTS:

- RNase A (Cat# M1227-25)
- RNaseOFF ribonuclease Inhibitor (Cat# M1238-4000)
- DNase, *E.coli* DNA Ligase (Cat# M1217-100)
- Link-FAST™ 5 Minutes DNA Ligation Kit (Cat# K902-50)
- New T4 DNA Ligase (Cat# M1247-200)
- T4 DNA Ligase (5 u/µl) (Cat# 9101-250)
- T4 RNA Ligase 1 (ssRNA Ligase) (Cat# M1218-100)
- T4 RNA Ligase 2 (dsRNA Ligase) (Cat# M1219-100)
- T4 RNA Ligase 2 (Truncated) (Cat# M1220-100)

FOR RESEARCH USE ONLY! Not to be used on humans.

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