ab286929 – RNase R

For the enrichment of circRNAs in biological samples, identification of intronic lariat sequences, identification of exonic circRNAs, studying alternative splicing, production of artificial circular RNAs.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab286929

Storage and Stability

On receipt entire assay kit should be stored at -20°C. Avoid repeated freeze-thaw cycles. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
RNase R (10 U/µL)	50 µL (500 U)	-20°C
10X RNase R Reaction Buffer	1 mL	-20°C

Enzyme 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.

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 (10 U/µL)	4 + 4 µL (supplemented partway)	2 µL
RNaseR Buffer [10x]	5 µL	2 µL
Ribonuclease Inhibitor [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.

Technical Support

For all technical or commercial enquiries please go to:

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