XRN-1

Product description

XRN-1 is a highly efficient exoribonuclease that strictly requires a 5' monophosphate (5'-p) substrate. In the presence of Mg^{2+}, it specifically digests 5'-monophosphorylated single-stranded RNA in the 5'→3' direction. XRN-1 also acts on 5'-monophosphorylated single-stranded DNA (ssDNA), albeit with significantly lower efficiency.

XRN-1 exhibits strict substrate specificity and cannot digest the following substrates: RNA bearing 5' triphosphate (5'-ppp), 5' diphosphate (5'-pp), 5' cap (5'-cap), or 5' hydroxyl (5'-OH) termini; double-stranded DNA (dsDNA); and ssDNA with 5'-OH, 5'-ppp, or 5'-pp ends. In addition, it does not cleave double-stranded RNA with recessed 5'-phosphorylated termini, DNA/RNA hybrids, or 5'-phosphorylated RNA sequestered within stable secondary structures.

This enzyme is commonly used in sequencing library construction for the separation and enrichment of mRNA from total RNA, the removal of template DNA and by-products from in vitro transcription products, and the analysis of the 5' end of RNA for research purposes.

Composition and storage conditions

Components \ Size	20 U	100 U	500 U	Storage
XRN-1 (1 U/μL)	20 μL	100 μL	500 μL	-20°C
10× XRN-1 Reaction Buffer	50 μL	250 μL	1.25 mL	-20°C
Shipping: Dry Ice	**Shelf life:** 2 years			

Experimental operation

1. Thaw reagents on ice and prepare the reaction mixture on ice as follows:

Reagent	Volume	
10× XRN-1 Reaction Buffer	2 μL	1×
Substrate (RNA Sample)	X μL	Up to 1 μg
XRN-1 (1 U/μL)	1 μL	
Nuclease-free Water	To 20 μL	

Total Reaction Volume	20 μL	

***Note:** RNA substrate must be dephosphorylated prior to use to generate 5'-P ends; otherwise, the enzyme activity will be extremely low. Strictly follow RNase-free practices for all reagents and consumables. Avoid inhibitors such as EDTA and SDS in the reaction. Excessive EDTA will inhibit the activity of XRN-1. Therefore, TE buffer containing EDTA cannot be used to dissolve RNA samples. It is recommended that the substrate RNA samples be dissolved in nuclease-free Water. Additionally, this reaction system has high requirements for the purity and quality of RNA samples. Poor RNA quality will affect the digestion reaction.

2. Gently mix the reaction (by pipetting or tapping the tube; do not vortex), then briefly centrifuge to collect contents.
3. Incubate at 30°C for 60 min (adjust time based on substrate length). Stop the reaction by heating at 65°C for 10 min.

***Note:** Extend incubation time if digestion is incomplete.

4. After incubation, mix samples 1:1 with 2X RNA Loading Buffer (Cat. No. K1163). Analyze by electrophoresis on 1-2% agarose gel or 15% urea (7M) polyacrylamide denaturing gel, according to fragment size.

Product properties

1. The amount of enzyme required to degrade 1 nmol of 5'-P RNA substrate per minute at 30°C.
2. Quality assurance:
   - High Specificity: Strictly recognizes 5'-P RNA; no activity toward 5'-ppp RNA or m^7G-capped RNA.
   - High Activity: Degrades thousands of nucleotides per minute in optimal buffer.
   - RNase-Free: Rigorously purified; free of endoribonuclease contamination.
   - High Purity: ≥95% purity by SDS-PAGE; free of DNase and phosphatase.
3. Heat Inactivation: 10 min at 70°C.

Notes

1. Keep the enzyme on ice during use and store at -20°C immediately after use. Additionally, EDTA >1 mM will inhibit the enzymatic activity.
2. If reducing enzyme amount, dilute in 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% glycerol. Do not vortex during dilution to preserve activity.
3. This product is for research use only.

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