RNase T1

REF: EG24210-S/M

Storage Condition

-20℃

Components

Component	EG24210S	EG24210M
RNase T1 (500 U/μl)	200 μl	5×200 μl
10× RNase T1 Buffer	1 ml	5×1 ml

Description

RNase T1 is an endoribonuclease obtained from Aspergillus oryzae, which exhibits specificity for cleaving single-stranded RNA following guanosine ribonucleotides (G), resulting in the formation of 3'-phosphate termini. RNase T1 is capable of forming a nucleoside 2', 3'-cyclic phosphate intermediate to cleave the phosphodiester bond between the 3'-guanosine residue and the 5'-OH group of the adjacent nucleoside, producing oligonucleotides with terminal 3'-GMP and 3'-GMP.

The activity of RNase T1 does not depend on metal ions.

Definition of Activity Unit

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm in 15 min when yeast RNA is hydrolyzed at 37℃ and pH7.5.

Applications

1. RNA removal from recombinant proteins.
2. RNA removal from recombinant proteins.
3. RNA sequencing.
4. RNA protection assay (in conjunction with RNase A).
5. Detecting the transcription level of G-free or low-G DNA templates.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 500 U of RNase T1 incubated for 4 hours at 37 ℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 500 U of RNase T1 incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

1. Reaction system

Reagent	Amount	Final Concentration
ssRNA	1~10 μg	50~500 ng/μl
10× RNase T1 Buffer	2 μl	1×
RNase Inhibitor (40 U/μl)*	1 μl	2 U/μl
RNase T1 (500 U/μl)	0.4 μl	10 U/μl
Nuclease-Free Water	up to 20 μl	-

Note: To prevent RNA degradation, it is recommended to add RNase Inhibitor (REF: EG20002) into the reaction mixture Substrate RNA should be added after the addition of RNase Inhibitor.

2. Recommended reaction conditions

Incubate at 37 ℃ for 15 minutes. The incubation time can be appropriately extended or shortened to ensure sufficient digestion.

Notice

1. Metal ions Mg^{2+} (100mM $MgCl_2$ inhibits about 40% activity), Ca^{2+} (10mM $CaCl_2$ inhibits about 30% activity), Zn^{2+}, Fe^{2+}, Cu^{2+} can inhibit the activity of RNase T1. Guanylyl 2'-5' guanosine is a specific inhibitor of RNase T1.
2. RNase T1 after heat-inactivation is reversible. It is recommended to remove RNase T1 by column purification or phenol/chloroform extraction.
3. RNase T1 is highly tolerant to heat. Under pH6.0, it can tolerate 100℃ for 10 minutes, but it is unstable in alkaline solutions with a pH > 9.0.
4. For your safety and health, please wear a lab coat, disposable glovesand a mask while conducting the experiment.

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