Tinzyme Co., Limited

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Thermostable RNase H

Product Number:RN02

Shipping and Storage

-20°C.

Description

Thermostable RNase H is an endonuclease that specifically hydrolyzes hybridization to DNA at high temperatures The phosphodiester bonds of the RNA on the strand, so it can degrade the RNA in the DNA/RNA hybrid strand, without digesting the single- or double-stranded DNA. Heat-resistant RNase H and RNase H in E. coli is a homologous protein and has endonuclease activity, but heat-resistant RNase H also exhibits activity at 65°C, which makes the enzyme Can be used for high temperature experiments. The enzyme gene is derived from the extreme thermophilus Thermus thermophilus, which is introduced into the E. coli plasmid and is expressed and purified.

Application

1. Removal of mRNA that is heterozygous with the first strand of cDNA when synthesizing the second strand of cDNA;
2. Remove mRNA poly(A) hybridized onto poly(dT);
3. Lysis probe method to detect mRNA capping rate.

Notes

Since the reaction buffer contains Mg2+, under high temperature reaction conditions, when the system contains RNA/DNA heterozygous strands, there are other single-stranded RNAs, the temperature and reaction time should be appropriately reduced to reduce the impact on single-stranded RNA.

Components

Components	volume
Thermostable RNase H (5U/μl)	50μl
10×Thermostable RNase H Reaction Buffer	1ml

Unit definition

At 45°C, in a 50μl reaction system,after 20 min the amount of enzyme required to be able to fully digest 1nmol [H3]-labeled Poly(rA) hybridized double-stranded RNA with Poly(dT) into ribonucleotides is defined as 1 active unit.

Quality control

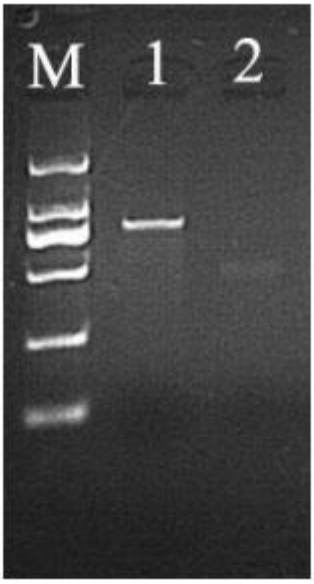
After multiple column purifications, only clear single band of interest was visible for SDS-PAGE glue detection, and there was no E. coli DNA residue and no nucleic acid internal and external nuclease contamination by PCR method.

Protocol

1. Digestion of DNA/RNA heterozygous duplexes

Components	Volume
DNA/RNA heterozygous strands	2μg
Thermostable RNase H	5U
10×Thermostable RNase H Reaction Buffer	10μl
RNase Free Water	Up to 100μl

Incubate at 45°C for 20 minutes, and after the reaction, 1μl of 0.5M EDTA can be used to terminate the reaction.



Lane1: DNA/RNA heterozygous double-stranded fragment is 810bp;

Lane2: DNA/RNA heterozygous double-strands are degraded by RNaseH and only single-stranded DNA remains.

2. Lysis probe method to detect mRNA capping rate

2.1. mRNA sample processing

Note: The following is an example of a biotin-labeled lysis probe.

Components	volume
Biotin-labeled lysis probes	500pmol
mRNA samples	100pmol
Thermostable RNase H	10μl
10×Thermostable RNase H Reaction Buffer	10μl
RNase Inhibitor, GMP Grade	3μl
RNase Free Water	Up to 100μl

The above mixture was mixed and placed in a PCR instrument, reacted at 50°C for 45min, and the lysis product was purified and tested on the machine.

Related products

Product Number	Product Name
M062	Vaccinia Capping Enzyme
GMP-RI01	RNase Inhibitor, GMP Grade
M072	mRNA Cap 2´ O Methyltransferase
RN01	RNase H, Recombinant