RNase H

Instruction for Use

Cat. No./Spec.: E1016-A/100 U, E1016-B/500 U

Concentration: 5 U/μL

Product Description

Ribonuclease H (RNase H) can specifically degrade the RNA strand within RNA-DNA hybrids. This enzyme does not hydrolyze the phosphodiester bonds in single-stranded or double-stranded DNA and RNA.

Components

Component	E1016-A	E1016-B
RNase H (5 U/μL)	20 μL	100 μL
10X RNase H Buffer	1 mL	1 mL

Storage Condition & Shelf Life

Store at -20°C.

Source

E. coli strain MRE-600.

Unit Definition

A unit is defined as the amount of enzyme required to catalyze the formation of 1 nmol of acid-soluble products within 20 minutes at 37° C.

Enzyme activity is determined in the following mixture: 20 mM Tris-HCl (pH 7.8), 40 mM KCl, 8 mM $MgCl_2$,1 mM DTT, 24 μM [^{3}H]-poly(A) ·poly(dT), 0.03 mg/mL BSA, and 4% (v/v) glycerol.

Scope of Application

- *Removal of mRNA before the synthesis of the second-strand cDNA*
- *RT-PCR and qRT-PCR: Removal of RNA after the first-strand cDNA synthesis*
- *Removal of poly(A) sequence from mRNA after hybridization with Oligo(dT)*
- *Site-specific cleavage of RNA*
- *Study of products from in vitro polyadenylation reactions*

Inhibition and Inactivation

- *Inhibitors: metal chelating agents, SH-blocking reagents.*
- *Heat at 65°C for 10 minutes.*

Protocol

Second-strand cDNA synthesis

1. Conduct the first-strand cDNA synthesis reaction according to the recommendations for the specific reverse transcriptase being used.

2. Add the following components (on ice) to the 20 μL first-strand cDNA synthesis reaction mixture:

Component	Amount
10X reaction buffer for DNA Polymerase I*	8 μL
RNase H (5 U/μL)	0.2 μL
DNA Polymerase I	30 U
ddH_2O	To 100 μL

** 10X reaction buffer for DNA Polymerase I: 500 mM Tris-HCl (pH 7.5 at 25 °C), 100 mM MgCl2, 10 mM DTT.*

③ Gently mix and briefly centrifuge.

④ Incubate at 15° C for 1 hour. Do not exceed the temperature of 15° C.

⑤ Add 2.5 μL (12.5 U) of T4 DNA Polymerase (#K011) and incubate at 15° C for 5 minutes.

⑥ Terminate the reaction by adding 5 μL of 0.5 M EDTA (pH 8.0). The blunt-end cDNA purified by phenol/chloroform can be used for further cloning-related procedures, such as adapter ligation, phosphorylation, size separation, ligation, and transformation.

This product is for research use only.