RNase H (RNase-free)

Introduce

RNase H is a ribonuclease enzyme that specifically hydrolyzes RNA phosphodiester bonds in DNA-RNA-binding chains. RNase H has a bivalent metal ion catalytic mechanism, such as $Mg2^{+}$ and $Mn2^{+}$ directly involved in the catalytic function. RNase H cannot hydrolyze phosphodiester bonds in single- or double-stranded DNA or RNA. This kit can be used to remove mRNA from the DNA and mRNA complex structures before the second strand of cDNA is synthesized, and to achieve pairing through complementary DNA sequences Spot-point snipping of RNA, identification of DNA-RNA hybrids, removal of poly(A) from mRNA hybridization onto poly (dT), In vitro polyadenylation reaction product studies.

Composition and storage conditions

Components / Size	500 U	1000 U	5000 U
RNase H (RNase-free)	0.1 ml	0.2 ml	1 ml
10X RNase H Reaction Buffer	0.5 ml	1 ml	1.5ml x2
Store at -20 °C.			

Experimental operation

1. Synthesize the first strand of cDNA with reverse transcriptase and terminate the reaction after incubating at 70 °C for 10 min.
2. Under the ice bath, add the following reagents to the above 20 μl reaction system in turn:

Component	Size
Reaction Buffer(10X)	2 μl
Nuclease-Free H_2O	17.8 μl
RNase H (5 U/μl)	0.2 μl

3. The system is ejected after mixing (pipette blowing or vortexing) and incubated at 37 °C for 1 h.
4. Add 2.5 μl of EDTA (pH 8.0) at a concentration of 0.5 M and mix well to terminate the reaction.
5. Subsequently, phenol chloroform extraction, ethanol precipitation, etc., or corresponding DNA purification kits can be used to purify the synthesized double-stranded cDNA.

Note: The pH range of *the RNase H reaction is about 7.5-8.3; This system is suitable for other similar DNA-RNA removal needs.*

Product information description

1. Definition of enzyme activity units:1 nmol is hydrolyzed from 100 bp of RNA-DNA hybrid chains within 20 min at 37 °C The amount of enzyme required in the acid soluble RNA form.
2. Liquid components in RNase H (RNase-free): 10 mM Tris-HCl (pH 7.4, 25°C), 50 mM KCl， 1 mM DTT, 0.1 mM EDT, 0.2mg/ml BSA, 50% Glycerol.
3. Buffer components in the 10X RNase H Reaction Buffer: 500 mM Tris-HCl (pH 8.3, 25°C), 750 mM KCl, 100 mM DTT, 30 mM $MgCl_2$.
4. RNase H quality and experimental validation:
   I. Protein purity > 95%.
   II. Verification of activity:
   - 5 U of RNase H and 200 ng of 300 base RNA substrate react at 37 °C for 16 h, RNA The electrophoresis band does not change.
   - 50 U of RNase H and 1 μg of supercoiled PhiX174 DNA react at 37 °C for 4 h, DNA The electrophoresis band does not change.
   - 50 U of RNase H and 1 μg of Hind III-λ DNA substrate were reacted at 37 °C for 30 min The electrophoretic bands of DNA do not change.
5. RNase H thermal inactivation conditions: Heating at 50 °C for 15 minutes can inactivate RNase H.