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DNaseI (RNase Free)

Cat. No. : CW2090S **(** 1000 U **)**

Shipping and Storage : Storage at -20°C

Components

Component	CW2090S (1000 U)
DNaseI (RNase Free),1 U/μL	1 mL
Reaction Buffer (with $MgCl_2$),10×	1 mL
200 mM EDTA	1 mL

Principle

DNase I is a divalent cation-requiring endodeoxyribonuclease that can be used to degrade single-or double-stranded DNA. The principle is that DNase I hydrolyzes phosphodiester bonds to generate mononucleotides or oligonucleotides with 5'-phosphate groups and 3'-OH. Either Mg^{2+} or Mn^{2+} can activate the activity of DNase I, while the concentration of Ca^{2+} directly affects the activity of the enzyme. In the presence of Mg^{2+}, cleavage can be randomly generated on each single strand of double-stranded DNA; while in the presence of Mn^{2+} double-stranded DNA can be broken. Mainly used for RNA preparation without DNA contamination, reverse transcription and in vitro transcription experiments.

Preparation before the experiment and important notes

1. Since DNase I is used in DNA digestion experiments that need to maintain the integrity of RNA, RNase contamination is avoided to the greatest extent during the enzyme preparation process and can be safely used for RNA extraction. However, since the enzyme does not contain RNase inhibitors, pay attention to prevent the contamination of exogenous RNase.
2. DNase I is greatly affected by shear force. Mix the centrifuge tube upside down before use, but avoid vortexing.
3. Do not use more than 1 U of DNase I per 1 μg of RNA processed.

Procedure

1. Taking the preparation of RNA samples for RT-PCR as an example, configure a 10 μL reaction system, as shown in the following table:

Component	Volume	Final concentration
RNA	X μL	1 μg
Reaction Buffer (with $MgCl_2$),10×	1 μL	1×
DNase Ⅰ （RNase Free）	1 μL	1 U
RNase Free Water	Y μL	Up to 10 μL

2. Incubate at 37°C for 30 minutes.
3. Add 1 μL of 200 mM EDTA and incubate at 65°C for 10 minutes to inactivate DNase I and stop the reaction.
4. The processed RNA can be used for RT-PCR.