Tinzyme Co., Limited

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Uracil-N-Glycosylase (UNG)

Product Number: UG01

Shipping and Storage

-20°C.

Components

Component	UG01 200U	UG01 1000U
Uracil-N-Glycosylase (UNG),1U/μL	200μL	1mL

Description

Uracil-N-glycosylase (UNGase), also known as uracil DNA glycosylase (UDGase), is a recombinant enzyme expressed and purified by E. coli. The protein has a molecular weight of 25 kD and can catalyze uracil-containing Single- and double-stranded DNA releases free uracil and is inactive to RNA, and is mainly used to prevent contamination of PCR amplification products. The mechanism of action of the enzyme is as follows: in the PCR reaction, dUTP is used instead of dTTP, and all Ts in the amplified product fragments are replaced by U, forming a PCR amplification product containing dU bases. UNG enzyme can selectively break the glycosidic bond of U base in single-stranded and double-stranded DNA, degrade the DNA containing U in the reaction system, effectively eliminate the residual contamination of PCR products, and greatly reduce false positives caused by contamination of amplification products, thereby ensuring specificity and accuracy of amplification.

Activity Definition

One unit is defined as the amount of enzyme required to catalyze 1nmol of uracil from uracil-containing DNA in 60 minutes at 37°C.

Quality Control

The purity detected by SDS-PAGE was more than 95%; no endonuclease or exonuclease activity was detected

Note

1. For long-term storage of UNG (infrequent use; less than 3 times a month), please store it at -70°C. For daily or weekly use, please store recommended to use in separate packaging for large packaging.
2. UNG can remove inadvertently contaminating U-DNA molecules before PCR reactions. A laboratory must use dUTP as one of dNTPs in all PCR reactions, so that all amplified products become U-DNA. If it is used alone for a certain detection, T-DNA will still accumulate, and this anti-contamination system is difficult to play a full role
3. The UNG/dUTP system is an anti-contamination measure inside the PCR reagent. In order to prevent the contamination of PCR products, especially when the same fragment is repeatedly amplified in the laboratory, the division and operation of the laboratory must be strictly regulated.

Protocol

The following example is the use method of Taq reaction system to prevent PCR product contamination, and the practical application can be improved and optimized according to specific experiments.

1. PCR Reaction System

Reagent	50μL Reaction System	Final Conc.
Taq PCR Buffer,10×	5μL	1×
Datp,10mM	1μL	200μM

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dGTP,10mM	1μL	200μM
dCTP,10mM	1μL	200μM
dTTP,10mM	0.5μL	100μM
dUTP,10mM	1μL	200μM
Forward Primer,10μM	1μL	0.2μM
Reverse Primer,10μM	1μL	0.2μM
Template DNA	XμL	
Taq DNA Polymerase,5 U/μL	0.5μL	2.5 U/50μL
Uracil-N-Glycosylase (UNG),1 U/ μL	0.2μL	0.2 U/50μL
ddH_2O	up to 50μL	

2. PCR Reaction Program

Step	Temperature	Time	
UNG digestion	37°C-50°C	5-10 min	
Predenaturation	95°C	10 min	
Denaturation	94°C	30 s	30-40cycles
Annealing	55-65°C	30 s	30-40cycles
Extend	72°C	1kb/min	30-40cycles
Final extension	72°C	5 min	

Note: Usually, Taq enzyme and UNG enzyme are added to the PCR reaction system in a certain proportion, firstly digested at 37°C-20°C for 5-10 minutes, and then inactivated at 95°C for 10 minutes. (At the same time, this step also achieves pre-denaturation. and hot-start effect) followed by PCR amplification. The reaction of UNG enzyme can be varied in the range of 37°C-50°C, 5-10 minutes, which can be adjusted according to the needs of the experiment.