

Temperature Sensitive UNG (TS-UNG)

Cat:U8170

Specification: 1U/ μ l

Storage: Store at -20°C. Avoid repeated freezing and thawing.

Introduction

TS-UNG Temperature Sensitive UNG is a temperature-sensitive UNG enzyme obtained through recombinant expression in E. coli. This enzyme can catalyze the release of free uracil from single- and double-stranded DNA containing uracil, and is inactive towards RNA. It is mainly used in PCR amplification to prevent product contamination. Compared to conventional UNG enzymes derived from E. coli genes, TS-UNG enzymes are temperature-sensitive and easily inactivated, avoiding the degradation of dUTP-containing amplification products at room temperature due to residual activity that may exist after inactivation of conventional UNG enzymes. TS-UNG enzymes can digest dUTP-containing templates at 20°C to 37°C for 5 to 10 minutes, and then inactivate at 50°C for 10 minutes or 95°C for 2 minutes.

Using the low-temperature digestion and easy inactivation characteristics of TS-UNG enzyme, it can be well matched with the RT-PCR amplification reaction procedure, thus being applied to reverse transcription amplification to prevent product contamination.

Definition of Activity: The quantity of enzyme that degrades 1 μ g of single-stranded DNA containing dU bases in 1 hour at 37°C is defined as 1 unit.

Storage Buffer: 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% glycerol, pH 7.5

Reaction Conditions:

1. Reaction System:

Component	Volume per Reaction(μ l)	Concentration in Master Mix
Template	*	< 1 μ g/reaction
Primer 1	1~5	0.2~1.0 μ M
Primer 2	1~5	0.2~1.0 μ M
dNTPs (dATP, dGTP, dCTP)	*	200 μ M
dUTP (replace dTTP)	*	400 μ M
10 \times PCR Buffer	5	1 \times
25mM MgCl ₂	2~8	1.0~4.0mM
Taq(5U/ μ l)	0.25	1.25U
TS-UNG(1U/ μ l)	0.5 (0.1-0.5)	0.5U (0.1~0.5U)
H ₂ O	*	—
Total volume	50 μ l	

2. Incubate the product for 10min at 37°C;

3. Inactivate TS-UNG by heating to 50°C for 10min, or 95°C for 2min;

Quality Control:

1. The purity of SDS-PAGE electrophoresis is greater than 98%;
2. Degradation activity, inter-batch variation, and stability;
3. No activity of exonuclease, endonuclease, and RNase;

Note:

1. Within the temperature range of 20°C to 37°C, the activity of the TS-UNG enzyme increases with rising temperatures. Reactions are typically conducted at 20°C, 25°C, and 37°C. The enzyme dosage can be optimized to range from 0.1 to 0.5U, and the reaction time can be adjusted to 5 to 10 minutes; It is especially suitable for RT-PCR to prevent contamination;
2. Due to the different utilization efficiency of dUTP and sensitivity to UNG enzyme for different genes to be amplified, if the use of UNG system leads to a decrease in detection sensitivity, the reaction system should be adjusted and optimized;
3. Avoid multiple freeze-thaw cycles and do not expose it to areas with large temperature fluctuations. Temperature fluctuations have a significant impact on product stability;
4. Before and after amplification, use dedicated areas and pipettes, wear gloves and change them frequently, and do not open the reaction tubes after the PCR reaction is complete. to minimize the contamination of PCR products on the sample.