Tinzyme Co., Limited

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Terminal deoxynucleotidyl transferase

Product Number: TDT01

Shipping and Storage

Ice pack transportation; Stored at -20°C, with a shelf life of 2 years, to avoid repeated freezing and thawing.

Components

Component	500U	2.5KU	10KU
TdT（20 U/μL）	25μL	125μL	1mL
5 × Reaction buffer	300μL	1.5mL	1.5mL×4

Description

Terminal Deoxyribonucleic Acid Transferase (TdT) is a template independent DNA polymerase that catalyzes the addition of dNTP to the 3 'hydroxyl end of oligonucleotides, single stranded or double stranded DNA. The shortest length of a catalytically active oligonucleotide is 3 nucleotides. There are reports that TdT can also add NTP to the 3 'hydroxyl end of RNA, but its catalytic activity on RNA is weaker than that on DNA.

Application

This product is widely used for oligonucleotide or DNA 3 'hydroxyl terminal labeling; DNA tailing at the end of the DNA; 5'-RACE； Synthesize oligomeric chains of the same deoxyribonucleotide, etc.

Product Information

1. Source: Expressed by Escherichia coli, the source of the expressed gene is calf thymus.
2. Unit definition: The amount of enzyme required to catalyze the addition of 1nmol dNTP to the 3 'hydroxyl terminus of a polynucleotide at 37°C for 60 minutes is defined as one active unit.
3. Inactivation or inhibition: Heating at 70°C for 10 minutes or adding an appropriate amount of EDTA can cause Terminal Deoxynucleotidyl Transferase to become inactive. Metal ion chelating agents, with higher concentrations of ammonium ions, chloride ions, iodide ions, and phosphate ions, all have inhibitory effects on Terminal Deoxynucleotidyl Transferase.

Protocol

1. Adding a tail to the end of DNA

1.1. Prepare the following mixture in an RNase free centrifuge tube:

Component	Volume
DNA fragments	1pmol of 3'-termini
5 × Reaction Buffer	4μL
dATP or dTTP or dGTP or dCTP (Usually only one)	130pmol (20μCi)
(3000Ci/mmol)	60 pmol
TdT（20 U/μL）	0.5-1.5μL
RNase-free ddH_2O	to 20μL

The prepared transposon can be used for fragmentation experiments and can also be stored at -20°C.

1.2. After setting up the reaction system according to the table above, gently mix it (you can use a pipette to blow and mix or use a Vortex to mix at the lowest speed), and then centrifuge to precipitate the liquid.

1.3. Incubate at 37°C for 20 minutes.

1.4. Incubate at 70°C for 20 minutes or add 5μL of 0.5M EDTA to terminate the reaction.

Note: Under the above reaction conditions, 100-130 dA or dT, or 20-30 dC or dG can be added to each 3 'hydroxyl end.

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2. DNA 3 'end marker

Prepare the following mixture in an RNase free centrifuge tube.

Component	Volume
DNA to be labeled	10 pmol of 3'-termini
5 × Reaction Buffer	10μL
[α-32P]-ddATP, ~10TBq/mmol	1.85MBq
(3000Ci/mmol)	(50μCi)
TdT（20U/μL）	1-2μL
RNase-free ddH_2O	To 50μL

2.1. After setting up the reaction system according to the table above, gently mix it (you can use a pipette to blow and mix or use a Vortex to mix at the lowest speed), and then centrifuge to precipitate the liquid.

2.2. Incubate at 37°C for 20 minutes.

2.3. Incubate at 70°C for 20 minutes or add 5μL of 0.5M EDTA to terminate the reaction.

Note: The efficiency of labeling is related to the type of 3 'hydroxyl end, and the labeling efficiency of 3' protruding end is significantly higher than that of 3 'recessed or flat end.

Note

1. This product is only for scientific research purposes.
2. Please keep the experimental area clean; Clean gloves and masks should be worn during operation.
3. Enzymes should be stored in an ice box or on an ice bath during use, and immediately stored at -20°C after use.