

## Product components

Components	Component number	Size-1	Size-2
		500 U	2500 U
Terminal Transferase (20,000 U/mL)	RM20535	25 $\mu$ L	125 $\mu$ L
10X Terminal Transferase Reaction Buffer	RM20805	1.5 mL	1.5 mL
2.5 mM CoCl <sub>2</sub>	RM20811	1.5 mL	1.5 mL

## Product Description

Terminal transferase (TdT) is a **template-independent** DNA polymerase that catalyzes the addition of deoxynucleotides to the 3' hydroxyl terminus of DNA molecules. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT.

**Its** main application is oligonucleotide or DNA 3' termination label; DNA tailing (DNA tailing); 5'-RACE; synthesis of oligomeric chains of the same deoxynucleotide, etc.

## Source

E.coli cells carrying a cloned gene encoding calf thymus terminal deoxynucleotidyl transferase.

## Store

-20°C

## Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 1 nmol of deoxythymidylate into a polynucleotide fraction in a total reaction volume of 50  $\mu$ l in 60 min at 37 °C.

## Storage Buffer

50 mM KPO<sub>4</sub>, 100 mM NaCl, 1.43 mM  $\beta$ -ME, 50% Glycerol, 0.1% Triton® X-100, pH 7.3 @ 25°C

## Reaction Buffer

10X Terminal Transferase Reaction Buffer: 200 mM Tris-Ac, PH=8.0, 500 mM KAC, 100 mM MgAC<sub>2</sub>

## Inhibition and Inactivation

Inactivated by heating at 70 °C for 10 min

## Applications

Addition of homopolymer tails to the 3' ends of DNA.

Labeling the 3' ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP).

5'-RACE (Rapid Amplification of cDNA Ends)

TUNEL assay (in situ localization of apoptosis).

## 注意事项

1. The enzyme should be placed on an ice. And then stored at -20°C immediately after use.
2. EDTA can inactivate Terminal Transferase.
3. These substances have an inhibitory effect on terminal transferase activity, such as metal ion chelating agents, higher concentrations of ammonium ions, chloride ions, iodine ions and phosphate ions.
4. 330 ng for 100 bp, 1  $\mu$ g for 300 bp, 10 pmols DNA ends.

## Protocol

### Protocol for tailing of DNA 3'-termini:

1. Prepare the following reaction mixture (50 µL Reaction)

Reagent	50 µL
10X Terminal Transferase Reaction Buffer*	5µL
2.5 mM CoCl <sub>2</sub>	5 µL
25 mM dNTP**	0.4 µL
Terminal Transferase ***	0.5 µL
DNA (5.0 pmols)	2 µL
ddH <sub>2</sub> O	up to 50 µL

**Note:** \*, After Reaction Buffer is melted, please wait for the solution to return to room temperature, shake and mix well before use.

**\*\***, Optional A/T/G/C tailing reaction.

**\*\*\***, Terminal Transferase should be added to the reaction system at the end.

2. Mix the reaction components and centrifuge briefly to collect the solution to the bottom of the tube.
3. Incubate the mixture at 37 °C for 30 min.
4. Stop the reaction by heating at 70 °C for 20 min